Evaluation of a lysophospholipid using two oils on performance, carcass composition and organ characteristics of broilers

by

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Summary

The aim of the study was to investigate the potential to decrease the apparent metabolizable energy (AME) by including a lysophospholipid in the diet of broiler chickens. There were two oils used in the trail: refined soya oil and an unsaturated blend of animal fats and vegetable oils. For each type of oil, three diets were formulated, the first with standard AME and the other two containing 0.25 MJ/kg less. One of the reduced diets included a lysophospholipid, Lysoforte Extend Dry (LEX), at an inclusion level of 500 g/ton. Two thousand, one hundred and twelve chicks were randomly allocated to six treatments, where each treatment was replicated sixteen times. The broiler chickens were raised until slaughter at day 35 of age. Both oils were chemically analysed before diets were formulated, their AME values were calculated using the Wiseman equation corrected for moisture, impurities and unsaponifiables (MIU). Results from the analysis showed that AME values for young broilers, 0-21 days of age, was 36.69 MJ/kg for soya oil and 30.78 MJ/kg for the blended oil, a difference of 5.91 MJ/kg or 16.1% lower. The AME for older birds of > 21 days was 37.66 for soya oil and 33.82 MJ/kg for the blended oil, which was a difference of 3.84 MJ/kg or 10.2% lower. The first phase of the study involved the effect of the decreased AME value and the addition of LEX on broiler production parameters; these parameters included body weight (BW), feed intake (FI), feed conversion ratio (FCR), average daily gain (ADG), protein efficiency ratio (PER) and European production efficiency factor (EPEF). No significant differences were observed for any parameter on soya oil where LEX was added except cumulative FI, while on the blended oil the only parameters that were significantly lower than the control was average BW and a higher FCR. These two parameters of the blended oil were also significantly lower than soya oil with additional LEX. The second part of the trial investigated the effect on the organ and carcass characteristics of broilers. After slaughter the dressing percentage, relative organ weights, relative carcass portion weights and breast muscle pH were measured. No significant differences were observed for any parameter on the relative organ weights of the blended oil treatments, however on the soya oil treatments, significant differences were observed for the gizzard, liver, spleen and the gizzard erosion score. The only significant differences observed between soya oil and the blended oil was the liver and spleen relative weights, of which both was significantly higher on soya oil with additional LEX. On carcass characteristics there were no significant differences observed for any parameter on the blended oil treatments and also between the blended oil and soya oil treatments both with LEX. The only significant difference on soya oil was a lower relative breast weight when LEX was added, no other significant effects were observed for the soya oil treatments. Overall the study indicated that when LEX is added with a decreased dietary energy, there are no adverse effects on normal broiler production parameters, organ or carcass parameters of broilers. This highlights the importance of using LEX in the broiler industry, where reducing dietary energy results in a

saving on the feed cost and ultimately results in an increased profitability within the broiler industry.

Opsomming

Die doel van die studie was om die potensiaal te ondersoek om die oënskynlike metaboliseerbare energie (AME) te verminder deur 'n lysofosfolipied in die dieet van braaikuikens by te voeg. Daar is twee olies in die studie gebruik, geraffineerde soja-olie en 'n onversadigde mengsel van diere vet en plantaardige olies. Vir elke soort olie is drie diëte geformuleer, die eerste met standaard AME en die ander twee wat 0.25 MJ / kg minder bevat. Een van die verminderde diëte bevat 'n lysofosfolipied, Lysoforte Extend Dry (LEX), met 'n insluiting van 500 g / ton. Twee duisend, een honderd en twaalf kuikens is sonder uitsoek toegeken aan ses behandelings, waar elke behandeling sestien keer herhaal is. Die braaikuikens is tot op die ouderdom van 35 dae grootgemaak en daarna geslag. Albei olies is chemies geanaliseer voordat diëte geformuleer is, en hul AME-waardes is bereken met behulp van die Wiseman-vergelyking, gekorrigeer vir vog, onsuiwerhede en onversoenbare middels (MIU). Resultate uit die analise het getoon dat AME-waardes vir jong braaikuikens, van 0-21 dae oud, 36.69 MJ / kg vir soja-olie en 30.78 MJ / kg vir die gemengde olie was, 'n verskil van 5.91 MJ / kg of 16.1% laer. Die AME vir ouer voëls van > 21 dae was 37,66 vir soja-olie en 33.82 MJ / kg vir die gemengde olie, wat 'n verskil van 3.84 MJ / kg of 10.2% laer was. Die eerste deel van die studie het die effek van die verlaagde AME-waarde en die toevoeging van LEX op braaikuikenproduksie parameters behels, hierdie parameters het liggaamsgewig (BW), voerinname (FI), voeromsetverhouding (FCR), gemiddelde daaglikse groei (ADG), proteïne-doeltreffendheidsverhouding (PER) en Europese produksiedoeltreffendheidsfaktor (EPEF) ingesluit. Geen merkwaardige verskille is waargeneem vir enige parameter op soja-olie waar LEX bygevoeg is nie, behalwe kumulatiewe FI, terwyl die enigste parameters wat aansienlik laer was as die kontrole op die gemengde olie, die gemiddelde BW en 'n hoër FCR was. Hierdie twee parameters van die gemengde olie was ook aansienlik laer as soja-olie met addisionele LEX. Die tweede deel van die proef het die effek op die orgaan- en karkaseienskappe van braaikuikens ondersoek. Na die braaikuikens geslag is, is die uitslag persentasie, relatiewe orgaangewigte, relatiewe karkas porsies en pH van die borsspier gemeet. Geen merkwaardige verskille is waargeneem vir enige parameter op die relatiewe orgaangewigte van die gemengde oliebehandelings nie, maar wel op die soja-oliebehandelings is merkwaardige verskille gevind ten opsigte van die spier-, lewer-, milt- en die spiermaag erosie telling. Die enigste merkwaardige verskille wat tussen soja-olie en die gemengde olie waargeneem is, was die lewer en milt se relatiewe orgaan gewig, waarvan albei aansienlik hoër was op soja-olie met addisionele LEX. Wat die eienskappe van die karkasse betref, was daar geen merkwaardige verskille waargeneem vir die parameter op die gemengde olie behandelings nie, en ook tussen die gemengde olie- en soja-oliebehandelings, beide met LEX nie. Die enigste betekenisvolle verskil op soja-olie was 'n laer relatiewe borsgewig waar LEX bygevoeg is. Geen ander merkwaardige verskille is waargeneem vir die

behandelings met soja-olie nie. In die algemeen het die studie aangedui dat wanneer LEX bygevoeg word met 'n verminderde voedingsenergie sonder enige nadelige effek op die normale braaikuiken produksie parameters, die orgaan en karkasparameters van braaikuikens het nie. Dit beklemtoon die belangrikheid van die gebruik van LEX in die braaikuikenbedryf, waar die vermindering van dieëtenergie 'n besparing op die voerkoste tot gevolg het en uiteindelik 'n verhoogde winsgewendheid binne die braaikuikenbedryf tot gevolg het.

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Preface

This thesis is presented as a compilation of five chapters.

Chapter 1	**General Introduction**
Chapter 2	**Literature review**
Chapter 3	**Research results** The effect of two different oil sources and addition of a lysophospholipid on production parameters of broiler chickens
Chapter 4	**Research results** The effect of two different oil sources and addition of a lysophospholipid on the organ and carcass characteristics of broiler chickens
Chapter 5	**General conclusions**

Abbreviations

SAPA	**South African Poultry Association**
AME	**Apparent metabolizable energy**
FA	**Fatty acid**
SFA	**Saturated fatty acid**
UFA	**Unsaturated fatty acid**
MUFA	**Mono unsaturated fatty acid**
PUFA	**Poly unsaturated fatty acid**
FFA	**Free fatty acid**
MIU	**Moisture, impurities and unsaponifiables**
NSP	**Non-starch polysaccharides**
FCR	**Feed conversion ratio**
ADG	**Average daily gain**
LEX	**Lysoforte extend dry**
MJ	**Megajoule**
kg	**Kilogram**
g	**Gram**
ppm	**Parts per million**
U/S	**Unsaturated to saturated ratio**
PER	**Protein efficiency ratio**
EPEF	**European production efficiency factor**
DM	**Dry matter**
h	**hours**
ANOVA	**Analysis of variance**

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Chapter 1
General introduction

The poultry industry in South Africa accounted for 65.3% of the locally produced animal protein in 2018, making it the largest agricultural sector in the country (SAPA, 2018). According to figures from the South African Poultry Association (SAPA), the poultry industry supplied 1,657,000 tons of poultry meat in 2018 (SAPA, 2018). At the moment poultry meat is the most affordable source of animal protein. Worldwide there is an increase in feed costs, this rise in feed costs increase production cost of poultry meat and this will be transmitted to the consumer. In order to mitigate the increased feed cost, it remains important to search for more cost-effective feed utilization techniques, without compromising on nutritive quality or profitability within the industry. Lipids provide the main source of energy to animals and have the highest caloric value among all nutrients (Zhao & Kim 2017). Lipids are added to broilers diets to obtain energy dense diets required by the modern broiler for optimal growth performance and achieving the industry standards (Blanch *et al*., 1996).

Lipids are water insoluble compounds whose digestion takes place in an aqueous environment in the small intestines through the synergistic action of bile salts and pancreatic lipase. Bile salts ensure the emulsification of dietary fats which allows pancreatic lipase to hydrolyse the triglycerides that are present on the water-oil interface. Bile salts play a major role in mixed micelle formation which are absorbed on the mucosa cells in the small intestines (Kroghdahl, 1985). Where lipids are added to broiler diets, the use of an exogenous emulsifier can improve the emulsion and micelle formation - this leads to an improved lipid digestion and productive performance (Jansen *et al*., 2015; Zampiga *et al*., 2016). In a study conducted by Melegy *et al*. (2010) on low nutrient density diets, it was demonstrated that lysophospholipids could be used to compensate for these low-density diets without affecting the birds' performance. Lysophospholipids are formed through the hydrolysis of the ester bond of phospholipids. This process results in a more improved emulsification of fat into smaller droplets which has a larger surface area for lipase enzyme to work on.

Lysophospholipids have a lower critical micelle concentration and form smaller micelles when compared to phospholipids (Reynier *et al*., 1985; Zubay, 1983; Zampiga *et al*., 2016). Lysophospholipids are important in animal nutrition as biosurfactants and with the lipophilic and hydrophilic properties they contain, helps with their role as biosurfactants when they are mixed with water and lipids. The addition of lysophospholipids to the diet shows an increased absorption and digestion of lipids in the young chick (Sugumar, 2012). The effectiveness of emulsifiers is

dependent on the composition of the supplemental fat which include chain length, position of the fatty acid, degree of saturation and the level of dietary fat (Dierick & Decuypere, 2004).

There are many inconsistent results from the use of lysophospholipids in broiler production; conflicting results were found for production parameters, organ and carcass characteristics of broilers. These results may be attributed to the variation of the diets, the lysophospholipid inclusion level as well as the inclusion level of fat in the diet.

Therefore, the objectives for this study was to investigate the addition of a lysophospholipid while reducing the dietary energy in broiler chickens. The effect of the lysophospholipid with a reduced apparent metabolizable energy (AME) value was evaluated on the growth performance of broilers and on the effect on organ and carcass characteristics of broilers.

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Chapter 2
Literature Review

2.1 Use of lipids in poultry diets

2.1.1 Introduction

The term lipids, fats and oils are used interchangeably, and they all describe a variety of compounds that are insoluble in water. Lipids, fats and oils are added to broiler diets to achieve energy dense diets as they have the highest caloric value of all macro ingredients used in broiler feed (Lehninger, *et al*., 2008). The modern broiler has been selected to grow fast with a lower feed conversion ratio, highlighting the importance of using more energy dense diets whilst the birds are required to utilize all the nutrients available in the feed (Svihus, 2014; Cherian, 2015). The net energy obtained from the metabolizable energy of feed available to the chicks is 90% for fat, 75% for carbohydrates and only 60% for proteins (Scott *et al*., 1982). The importance of including fats and oils in the broiler diet has many more advantages than just increasing the energy density of the diet. These advantages include supply of essential fatty acids (FA) as birds are not able to synthesize all FA; dietary fat is also the major source and carrier of fat-soluble vitamins (A, D, E and K); it further results in improved pellet quality through lubrification of equipment and reduces heat increment of the feed (Murgeson, 2013).

2.1.2 Definition of Lipids

There are many possible definitions available for lipids and although there is no agreement on the exact definition of a lipid, the definition accepted for this dissertation is the following: Lipids are fatty acids and their derivatives (e.g. triglycerides) and substances related biosynthetically (e.g. lipoproteins) or functionally to these compounds (e.g. cholesterol) (AOCS, 2015). At room temperature fat is usually in a solid state, while the term oil refers to the esters of glycerol - oils are normally in a liquid state at room temperature (Baião & Lara, 2005).

2.1.3 Composition of lipids

The main constituent of fats and oils is a triglyceride (triacylglycerol). A triglyceride is formed by combining a glycerol with three molecules of fatty acid (FA) (Figure 2.1). The glycerol molecule

has three hydroxyl groups while each fatty acid has one carboxyl group. Ester bonds in triglycerides are formed from joining of the hydroxyl groups of the glycerol with the carboxyl groups of the FA.

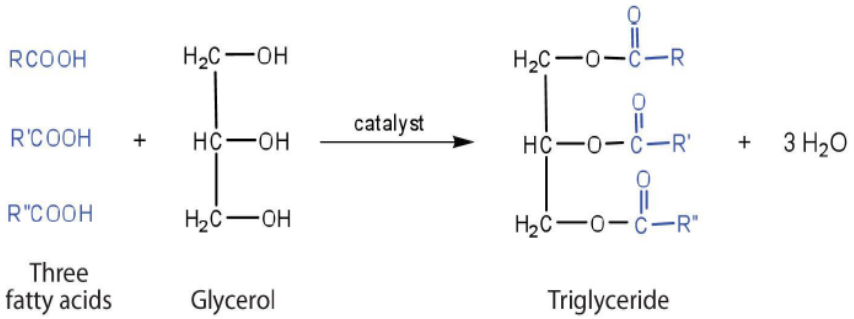


Figure 2.1 Structure of triglycerides. Hydrocarbon chains of the fatty acids are represented by R, R' and R'' (Ball *et al*., 2011).

The FA composition differs between different fat sources. These FA can be either saturated (animal fats) or unsaturated (most vegetable fats, with proportion of linoleic and in certain instances, also linolenic acid up to 60%) as showed in Figure 2.2. Saturated fatty acids (SFA) and unsaturated fatty acids (UFA) differ in the presence or absence of double bonds on the carbon chain. The SFA have no double bonds whereas UFA have one or more double bonds. Longer chains have fewer double bonds and they are also less soluble in water (Murugesan, 2013). A FA with only one double bond is known as mono-unsaturated fatty acid (MUFA) and FA with more than one double bond are known as poly-unsaturated fatty acids (PUFA) (Zimmerman & Snow, 2012; White, 2009). Fish oils mainly consists of PUFA whilst MUFA occurs more regularly in certain animal fats (Smink, 2012)

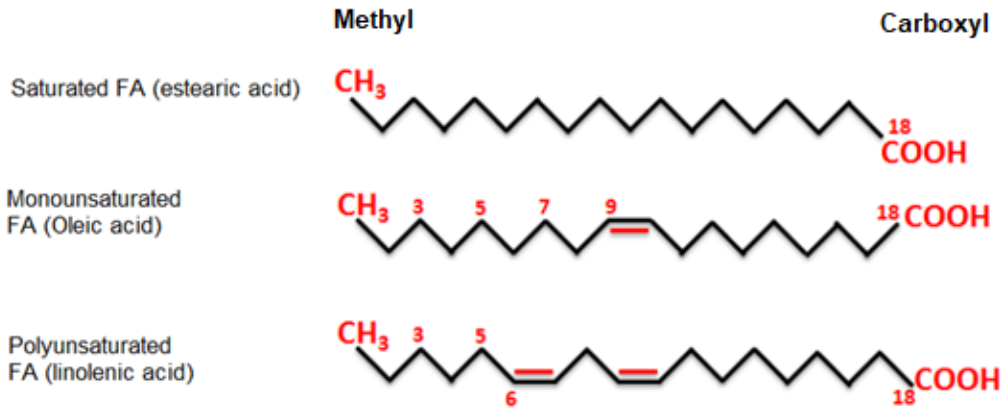


Figure 2.2 Structures of common saturated, monounsaturated and polyunsaturated fatty acids (Valenzuela & Valenzuela, 2013).

Fatty acids differ in the length of the hydrocarbon chain and can be divided into three categories: short chain fatty acids (hydrocarbon chains of less than eight carbon atoms), medium chain fatty acids (hydrocarbon chain consisting of eight to twelve carbon atoms) and long chain fatty acids (hydrocarbon chain consists of more than 12 carbon atoms) as shown in Table 2.1 below. The double bond found in MUFA as well as PUFA can be either in trans or cis configuration, depending on the position of the hydrogen atoms. A trans configuration is when the two hydrogen atoms are on opposite sides of the double bond. A cis configuration is when the two hydrogen atoms are on the same side as the double bond (Babayan, 1987, Fahy *et al*., 2005).

Table 2.1 Overview of the most common fatty acids found in fats and oils (adapted from AOCS, 2015)

Abbreviated designation	Fatty acid name	Carbon Atoms	Chain length
C4:0	Butyric acid	4	short
C6:0	Caproic acid	6	short
C8:0	Caprylic acid	8	short
C10:0	Capric acid	10	medium
C12:0	Lauric acid	12	medium
C14:0	Myristic acid	14	medium
C16:0	Palmitic acid	16	medium
C16:1	Palmitoleic acid	16	long
C18:0	Stearic acid	18	long
C18:1	Oleic acid	18	long
C18:2	Linoleic acid	18	long
C18:3	α-Linolenic acid	18	long
C20:0	Arachidic acid	20	long
C20:4	Arachidonic acid	20	long
C20:5	Eicosapentaenoic acid	20	long
C22:1	Erucic acid	22	long
C22:6	Docosahexaenoic acid	22	long

2.1.4 Digestion of lipids

Once a triglyceride is ingested, the digestion process occurs in the following three steps:
1. The bonds between the glycerol and two FA at C1 and C3 are hydrolysed by a lipase enzyme, which leaves two free fatty acids (FFA) and one monoglyceride.

2. Bile salts facilitate micelle formation of FA and monoglycerides which occurs within the small intestines.
3. Newly formed micelles move towards the intestinal wall; this occurs mostly in the jejunum portion of the small intestines, which is where the exchange occurs and FA as well as monoglycerides are absorbed. In the enterocyte, re-esterification occurs, and chylomicrons are formed and drain into the lymphatic vessels from the intestinal wall (Smink, 2012).

The main enzymes involved in lipid hydrolysis are lipases, phospholipase and cholesterolesterases. Lipase is mainly produced in the pancreas and is involved in splitting of the FA at the first and third position of the glycerol molecule, resulting in the formation of two FFA and one monoglyceride. Lipase enzymes have a high affinity for short and medium-chain FA and its activity is positively affected by bile salts and colipase. Phospholipase enzymes split the second position of the glycerol molecule, which creates lysophospholipids.

The process of fat hydrolysis results in bile acid micelles as end products. These micelles develop through an interaction with bile salt and other amphipathic products (fatty acids with a hydrophobic and a hydrophilic part) such as monoglycerides, medium-chain fatty acids, unsaturated fatty acids and lecithin. This leads to swelling of micelles, which creates space for hydrophobic products (diglycerides, long-chain SFA and fat-soluble vitamins) within the micelles. The required bile is produced in the liver and stored in the gallbladder. The concentration of bile salts in the intestinal contents should exceed 2 mmol bile/L, as a lower concentration will hamper micelle formation (Argenzio, 1984). This concentration is commonly referred to as the critical micelle concentration. The critical micelle concentration decreases with a higher concentration of monoglycerides (Freeman, 1984). The particle size of formed micelles is small enough to pass between the microvilli of mucosal cells. Fat absorption occurs between the end of the duodenum and the end of the ileum in monogastric animals, while absorption in the caeca and large intestines are negligible (Renner, 1965; Freeman, 1976; Kroghdahl, 1984). Bile salt in the micelles will be absorbed *via* an active and passive transport mechanism of which approximately 95% will be re-used. This re-using of bile salts plays an important role in digestibility of fat. The main site of lipid absorption is the proximal part of the jejunum. The FA and monoglycerides are re-synthesized into triglycerides within the mucosal cells where they are coated with protein (chylomicrons) and transported into the portal vein or *via* the lymph. Chickens absorb the fat directly into the portal vein (Krogdahl, 1985). This method for fat transportion in mammals is only for short and medium-chain fatty acids *via* the portal vein. The long-chain fatty acids are transported *via* the lymph.

2.2 Factors Influencing digestibility of fat sources

2.2.1 Introduction

There are several factors that could influence lipid digestion in broilers; these could be either related to animal characteristics or diet composition. Animal characteristics include factors such as age of the bird (Krogdahl, 1985; Tancharoenrat *et al*., 2013), genetic strain (Katongole & March, 1980), secretion and activity of the digestive enzymes (Nitsan *et al*., 1991; Nir *et al*., 1993; Noy & Sklan, 1995) and micro flora status (Maisonnier *et al*., 2003). Diet composition factors that impact lipid digestibility are type of fat used (Tancharoenrat *et al*., 2014), the ratio of saturated and unsaturated fatty acids (FA) (Wiseman, 1990) and amount and type of dietary fibre (Jimenez-Moreno *et al*. 2009).

2.2.2 Animal characteristics

2.2.2.1 Age

The chemical composition and nutritional values of all feed raw materials are published in the Central Bureau of Livestock Feeding (CVB) in the Netherlands. The CVB feed evaluation system is science-based and the main activities are:

- Data collection on chemical composition of feedstuffs and feed materials
- Collection of data on digestibility of feedstuffs for different farm animal categories
- Development and updating of feed evaluation systems for farm animals, and of energy and nutrient requirements.

According to Central Bureau of Livestock Feeding (2012) protocols for fat digestion, measurements are required at an age of approximately four weeks for broiler chickens. The digestibility is lower in younger animals as lipid metabolism is not yet fully developed in young birds (Krogdahl, 1985; Wiseman, 1990; Baiao & Lara, 2005; Tancharoenrat *et al*., 2013). Bile salts concentration is the first limiting factor followed by lipase secretion (Krogdahl, 1985; Ketels, 1994; Roy *et al*., 2005). The lowered lipid utilization in young birds is attributed to low bile salt concentration, which is caused by lowered synthesis of bile salts (Krogdahl, 1985; Meng *et al*., 2005). Krogdahl (1985) observed that through dietary supplementation of bile salts, lipid utilization can be improved.

Results of a study in broilers fed with two different fats at an age between two and eight weeks are presented in Table 2.2, which also shows the difference between unsaturated fat source (soybean oil) and saturated fat source (tallow) and their differences in digestibility. Table 2.3 illustrates how lipid digestibility increases with age of the bird. Between week one and week two the main increase was from 53.2% up to 80.7%.

Table 2.2 Faecal digestibility (%) of soybean oil and tallow in broilers at different ages (Ketels, 1994)

Age (weeks)	Soybean Oil	Tallow
2	75	42
3	87	53
4	92	63
8	91	67

Table 2.3 Fat digestibility in broilers at different ages (adapted from Tanchoenrat *et al*., 2013)

Age (weeks)	Fat digestibility %
1	53.2
2	80.7
3	85.9
5	85.7

Saturated fat sources are poorly digested by broilers when compared with laying hens, pigs and veal calves (Smink, 2012). The ability of the young chicken to digest long-chain saturated fatty acids, especially C16:0 and C18:0 is rather low. Addition of bile salts increased the digestibility of C16:0 and C18:0 by 2% points (Kussaibati *et al.*, 1982). There were no effects of the bile salt on the digestibility of unsaturated fatty acids C18:1 and C18:2 (Smink, 2012). Increasing the intake of saturated fatty acid sources decreases their digestibility (Ketels, 1994), indicating that the capacity for digestion of fat can easily be exceeded in young birds.

2.2.2.2 Gender and genetic strain

Lipid digestibility was found to be higher in female broilers (Guirguis, 1975). Females are able to deposit more body fat than males and also have an increased amount of abdominal fat. These differences can be as a result of different metabolism, greater competition between males, different capacities for fat accumulation, different nutritional needs and greater impact of

hormones in females (Tumova & Teimouri, 2010). It was observed by Slinger *et al*. (1955), that male broilers had a better growth performance over female broilers due to their superior ability for lipid digestion. Similarly, it was observed that male broilers have a higher growth rate and feed efficiency than female broilers (Becker *et al.*, 1981; Shalev & Pasternak, 1998; Huang *et al.*, 2008; Abdullah *et al.*, 2010). Contradictory to these results, Zelenka (1997) and Yaghobfar (2001), observed no difference between male and female broilers in their ability to digest lipids. The effect of broiler strain is also not clear-cut and is mainly attributed to genetic variation and not to the nutrient digestibility and absorption when varying results have been observed. Grunder *et al.* (1987) and Huang *et al.* (2008) showed a difference between broiler strains for abdominal fat deposition, while the contrary was observed by Becker *et al.* (1981) and Sonaiya & Benyi (1983) who observed no differences. With continuous genetic selection, further studies will be required to investigate gender and strain effects on lipid metabolism in broilers.

2.2.2.3 Microbiota

Dietary lipids can alter the microbial community (microbiota) of broilers (Knarreborg *et al*., 2002; Yang *et al*., 2009; Van der Hoeven-Hangoor *et al*., 2013) which will affect lipid digestibility. Despite several benefits to the host, the microbiota can result in detrimental effects under certain conditions. The microbiota can lead to a decrease in fat digestibility through deconjugating bile salts (Gaskins, 2001; van der Klis & Jansman, 2002). Bile salts are required to emulsify and absorb fat in the intestine. Catabolism of the bile salts in the gut by a variety of microbiota leads to a decrease in lipid absorption and results in the production of toxic products that inhibit the growth of chicken (Yadav & Jha, 2019).

2.2.3 Diet related factors

2.2.3.1 Lipid quality and inclusion level

Degree of saturation and chain length of fatty acids

The various lipid sources are not all equally utilized by the bird. The following factors can influence the utilization of lipids: degree of saturation, chemical structure of the lipid, carbon chain length and the oxidative state of the lipid (Renner & Hill, 1961; Freeman, 1984; Krogdahl, 1985; Baião & Lara, 2005). Saturated fatty acids, especially long chain FA have a lower digestibility and absorption rate in broilers when compared to short chain FA, medium chain FA and unsaturated FA. Tancharoenrat & Ravindran (2014) showed that oleic and linoleic acids (unsaturated FA) were digested and absorbed better than stearic acid (saturated FA). In the presence of

unsaturated FA there is a synergistic effect resulting in the improved digestibility of saturated FA and this has led to the use of blends of unsaturated and saturated lipid sources (Baião & Lara, 2005; Leeson & Summers, 2005; Tancharoenrat *et al*., 2013). Plant lipid sources have a higher unsaturated to saturated ratio than animal fats and are thus better utilized by the bird.

Free fatty acids

Free fatty acids (FFA) are formed as a by-product of lipid digestion and high levels are generally seen in by-product oils and restaurant greases. Free fatty acids negatively influence micelle formation and bile secretion, which results in decreased lipid digestibility as well as a lowered metabolizable energy (ME) (Freeman, 1976; Sklan, 1979; Wiseman *et al*., 1991). Wiseman & Salvador (1991) evaluated the effect of free fatty acids (FFA) content of three fat sources (Tallow, palm oil and soya oil) and showed a decrease in apparent metabolizable energy (AME) as the FFA increased, regardless of fat source.

Rancidity and oxidation

Oxidation is a degradation process which occurs at the double bond sites (unsaturated FA) in the glyceride molecules. These glyceride molecules are the building blocks of edible lipids. Lipids are more susceptible to oxidative breakdown with an increased number of double bonds. The first step in the oxidation process is the formation of a free fatty radical when the hydrogen is removed from the unsaturated FA group of the fat molecule. In the presence of atmospheric oxygen this free radical is susceptible to attack to form an unstable peroxide free radical. These free radicals are strong initiators and promoters of further oxidation (Sherwin, 1978). Oxidative rancidity results in decreased lipid quality, rancid odour, whilst the product colour is also affected, decreased palatability due to off flavours and a lowered nutritive value of the lipid (Baião & Lara, 2005). Oxidation can negatively affect the energy value of fats and oils. Jensen *et al.* (1997) demonstrated the negative effects of oxidized lipids on animal performance and a lowered meat quality; they reported the reason for decreased performance was due to a reduced feed intake because of reduced palatability of the feed.

Lipid inclusion level

Increasing lipid inclusion levels in poultry diets lead to a decreased lipid digestibility, this is due to the limited availability of lipase and bile salts for the increasing amounts of lipid. This is more pronounced in young broilers (Krogdahl, 1985; Wiseman *et. al.*, 1991; Blanch *et al*., 1996; Sanz *et al.*, 2000; Villaverde *et al.*, 2006; Smink *et al.*, 2010). Inclusion of lipids in broiler diets during the first week, promotes a better performance until 21 days (Freitas, 1999). In order to optimize lipid digestion a minimum level of 10 g/kg lipid, is necessary in the diet (Leeson & Summers,

2005). Cancado (1999) reported that birds receiving lipids in their diet, showed a higher apparent digestibility of fat than the birds receiving no lipids.

Moisture, impurities and unsaponifiables

Moisture, impurities and unsaponifiables (MIU) are diluting factors with no benefit for the bird. The maximum accepted level for moisture of fats and oils is 1.0%, as moisture interferes directly with the energy content of fat. Impurity is the percentage of the insoluble fraction of the fat in petroleum ether and the content should be below 1%. Unsaponifiable matter, which includes steroids, pigments and hydrocarbons, form soaps when mixed with caustic soda. These substances are indigestible and are soluble in common solvents for oils. Therefore, an increase in unsaponifiable matter will result in a lower energy value of the fat or oil. The maximum level of unsaponifiable matter admitted in oils and fats is also 1%. (Butolo, 2002; Baião & Lara, 2005).

2.2.3.2 Dietary calcium levels

The hydrolysis of triacylglycerides forms monoglycerides and FFA, these FFA can react with other nutrients to form soluble and insoluble soaps. Insoluble soaps cause the FA and the mineral that it's bound to, to be unavailable to the animal (Leeson & Summers, 2005). Tancharoenrat & Ravindran (2014) identified calcium-phytate as a substrate during the formation of insoluble metallic soaps in the gastrointestinal tract of broilers. Dietary calcium level and type of fatty acid impacts calcium metabolism and soap formation. Atteh & Leeson (1983) fed broilers different supplemental FA using two different levels of calcium in the diet. The results showed that increasing calcium levels led to a reduction of lipid retention in birds fed palmitic acid, while the FA type affected calcium retention with palmitic and stearic acid resulting in a lower retention than the unsaturated FA. Tancharoenrat & Ravindran (2014) investigated the effect of three levels of dietary calcium with three inclusion levels of tallow on fat digestibility. The results again showed that with an increase in calcium there was an increased calcium soap formation and a decrease in calcium as well as fat digestibility.

2.2.3.3 Non-starch polysaccharides

In broilers, wheat, barley and cereal by-products are often used as a replacement of maize in the diet and this impacts fat digestibility (Smink, 2012). It is known that the carbohydrate sources with specific non starch polysaccharides (NSP) as found in rye, barley and wheat are known to reduce lipid digestibility and exhibit anti-nutritional effects in poultry (Choct & Annison, 1992; Lee *et al*., 2004; Meng *et al*., 2005; Smeets, 2015). Inclusion of a viscous water-soluble NSP in the diet,

resulted in increased microbial activity within the small intestine, which in turn will lead to degradation of bile acids, resulting in less effective fat emulsification (Smink, 2012). Langhout (1998) showed that an increased microbial activity in the small intestine will increase the deconjugation and excretion of bile salts with the droppings. Enzymes for the breakdown of water-soluble polysaccharides found in wheat, barley and rye will improve the digestibility of fat (Langhout, 1998; Dänicke *et al.*, 2000).

2.3 Fat and oil sources used in broiler diets

In broiler nutrition, a variety of oil and fat sources are used as energy source within the diet. Vegetable oils have a high metabolizable energy value due to the higher content of unsaturated fatty acids, unlike animal fats, which contain higher amounts of saturated fatty acids (Murgeson, 2013). The results obtained by Moura (2013), showed that when oil was included in broiler rations, they had an improved performance compared to broilers fed rations without oil.

2.3.1 Vegetable oils

2.3.1.1 Cotton oil

The use of cotton oil is limited due to the presence of gossypol, which is a toxic and anti-nutritional element. Ferrous sulphate must be added to the diet when cotton oil is used as it chelates gossypol which prevents its absorption in the digestive tract and neutralizes the effect. Broilers can tolerate levels up to 100 ppm of free gossypol without any effect on their performance (Baiao & Lara, 2005). In trials by AbdalQadir *et al*. (2014), four levels of cotton seed oil were used (0, 3, 6 and 9%); the final live weight at 50 days was significantly higher for the 0, 3 and 6% group. Similarly, as pertaining to the feed conversion ratio, the 0% group was significantly lower than the 3 and 9% group.

2.3.1.2 Canola oil

Rapeseeds that contain less than 2% euric acid in relation to the total fatty acid and less than 30 µmoles of glucosinate per gram of free oil on seed dry matter basis is called canola oil (Leeson & Summers, 2001). Thacker *et al*. (1994) observed that female broilers fed diets containing two different forms of canola oil had a higher growth rate than female broilers receiving diets with only tallow. These improved growth rates are due to a higher percentage of long chain fatty acids and an increased content of triglycerides within canola oil (Thacker *et al*., 1994). Similar results were

observed when carcass yield and cut yields of broilers were compared using canola oil, sunflower oil, corn oil, soybean oil and pig lard (Andreotti *et al*., 2001).

2.3.1.3 Sunflower oil

Alao & Balnave (1984) reported that broilers had an improved feed conversion ratio as well as better development when receiving diets containing sunflower oil compared to broilers receiving olive oil in their diets. It was suggested that this difference was due to the difference in fatty acid composition between the two vegetable oils. Sanz *et al*. (2000) evaluated two lipid sources, beef tallow (saturated fatty acid) and sunflower oil (unsaturated fatty acid) at inclusion levels of 8%. The birds fed diets containing sunflower oil had significantly reduced abdominal fat. The utilization of an unsaturated lipid reduces fat and results in an increase in carcass protein as the energy derived from unsaturated fat may be used for other metabolic purposes, while energy derived from saturated fat sources is not well utilized and thus accumulates as body fat (Sanz *et al*., 2000[b]). Using this reason from Sanz *et al*. (2000[b]), it can be concluded that the sunflower oil is better utilized compared to beef tallow.

2.3.1.4 Linseed oil

Lopez-Ferrer *et al*. (1999) test the effect of linseed oil, soybean oil, canola oil and sunflower oil on the nutritive and organoleptic traits of the meat and fatty acid profiles of five-week-old broilers. The results for meat quality showed no significant difference for linseed oil compared to the other oils, while the abdominal fat and breast muscle contained higher levels of omega-3 in the birds fed linseed oil in their diets.

2.3.1.5 Palm oil

Palm oil or mixtures of palm oil are fatty acids that have been distilled from palm and calcic soaps. They are classified as a vegetable oil with a fatty acid profile that can replace animal fats without having a significant impact on broiler carcass quality (Rodriguez *et al*., 2002).

2.3.1.6 Degummed soybean oil

Raw soybean oil has several substances considered as impurities that need to be removed through filtration, hydration and degumming. The substances from the extraction process are solid residues and include: Phospholipids, gums, metallic complexes, free fatty acids, peroxides,

polymers, secondary products from oxidation and pigments (Beauregard *et al*., 1996). Female broilers were fed with rations containing beef tallow, soybean oil, canola oil, fish oil, or a mixture of these oils. The chicks receiving soybean oil had significantly higher live weight (Scaife *et al*., 1994). Broiler rations containing 0, 4 and 8% soybean oil or acidulated soybean oil soapstock showed similar weight gain, however the feed conversion ratio was significantly improved with soybean oil. When the oil inclusion was increased from 4% to 8% there was a significant reduction in feed intake for the rations containing acidulated soybean oil soapstock, however this was not observed in the soybean oil treatments (Vieira *et al*., 2002).

2.3.2 Animal Fats

2.3.2.1 Poultry fat

Poultry Fat is the component remaining after solids and moisture is extracted through the normal rendering process. Also known as *viscera* oil and is derived through a process of extraction of fat by autoclaving or in a percolator tank with an expeller. After extraction, the fat is placed in a decanting tank to extract the excess moisture and acidulated to form soapstock. At this point the poultry fat is ready to be used in feed (Neto, 1994). The yield varies from 1.3% to 1.6% of liveweight depending on level and source of energy used in the ration, sex, age and weight of bird at slaughter (Mano *et al*., 1999). Diets containing either 4% poultry fat, 4% soybean oil or a mixture of 2% poultry fat and 2% soybean oil, showed no effect on weight gain, feed intake or feed conversion ratio. There was however a decreased feed intake and weight gain when the mixture was used (Dutra Jr *et al*., 1991). It was observed by Lara *et al*. (2003), that when different lipid sources (raw soybean oil, poultry fat and acidulated soybean oil soapstock and their mixtures) were evaluated for performance parameters, there were no significant differences observed for weight gain, feed intake, feed conversion ratio or viability on both the soybean oil as well as poultry fat rations.

2.3.2.2 Beef tallow

When 8% of sunflower oil, fish oil or beef tallow were added to broiler diets, it was observed that the rations containing beef tallow had the lowest feed conversion ratio (Newman *et al*., 2002). When sunflower oil was compared to beef tallow and pig lard by Sanz *et al*. (1999), the saturated fats (beef tallow and pig lard) resulted in a higher accumulation of intramuscular fat and abdominal fat. It was confirmed in another trail where 8% of sunflower oil and 8% of beef tallow were compared, the result was a significant reduction of abdominal fat in the diets where sunflower oil

was used (Sanz *et al*., 2000[b]). This is due to the energy derived from beef tallow being less promptly utilized and thus stored as body fat. There are also problems related to feeding tallow to broilers. The most notable of these result in the so-called oily bird syndrome. In experiments by Jensen *et al*. (2013), feeding a diet with a more saturated fat (tallow) to chicks from three to seven weeks of age, resulted in an increased incidence of oily bird syndrome over that of birds fed a more unsaturated fat (poultry oil). Oily bird syndrome results in broiler carcasses that are oily and greasy to the touch, and often have pockets of water accumulating in regions beneath the skin. Characteristics of oily bird syndrome are caused by changes in skin collagen structure. The various skin layers separate more easily, and oil and/or chilled water accumulates in the discreet pockets, especially in the back region (Summers *et al*., 2013).

2.3.2.3 Pig lard

Andreotti *et al*. (2001) conducted a trial using poultry fat, refined soybean oil, refined canola oil, refined sunflower oil, refined corn oil and pig lard on broilers from day 21 until day 49 and observed that there were no effects on performance parameters between the lipid sources. Confirming these results, Fébel *et al*. (2008) reported no significant differences in growth performance when sunflower oil and lard were used in broiler diets.

2.3.2.4 Fish oil

The production of fish oil comprises the compression of whole fish and sub products of the fishing industry. The oil produced is high in long chain polyunsaturated fatty acids, which can result in oxidative instability as well as transferring of the fish flavour onto the meat of the animals fed fish oil. Fish oils are generally high in omega-3, but low in omega-6 and linoleic acid. There is variation in the fatty acid profile of the fish oil as it can be influenced through period of fishing, processing method and dominant fish species caught/included (Fedna, 1999). An unpleasant fish taste of the broiler meat was observed at inclusion levels of 1.5% to 2.5% of fish oil (Hardin *et al*., 1964; Miller & Robisch, 1969) although an inclusion of 8% of fish oil in broiler diets resulted in a decreased carcass fat and an improved feed conversion ratio (Newman *et al*., 2002).

2.4 Fat emulsifiers

Due to the insolubility of lipids in water, emulsification is required before the lipolytic enzymes can commence with digestion. Emulsification is dependent on the lipid characteristics which includes chain length, FA positioning and saturation (Jansen, 2015). Enhanced emulsification is seen with

lysophospholipids, increasing their importance for oil in water emulsions within the gastro-intestinal tract, as demonstrated for phospholipids in Figure 2.3. Lysophospholipids are more hydrophilic than phospholipids due to the presence of only one FA residue on the molecule compared to phospholipids which contains two FA (Figure 2.4).

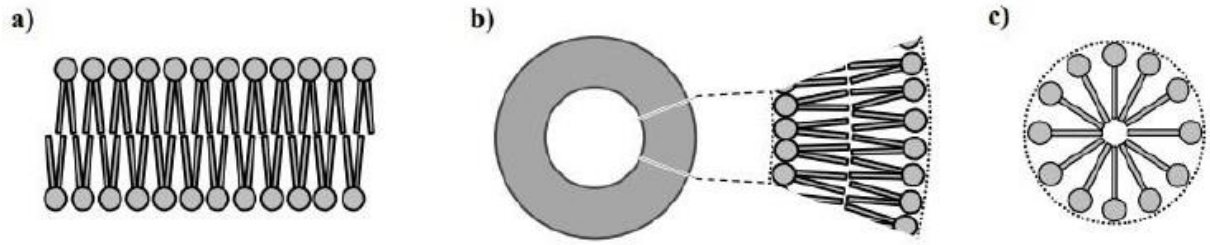
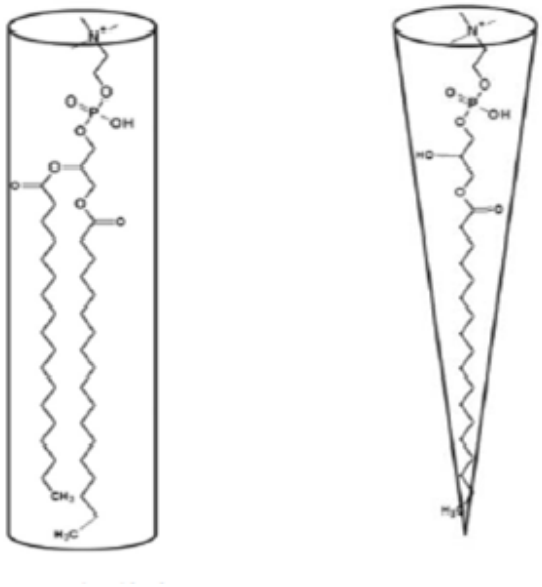


Figure 2.3 Illustration of the assembly of phospholipids and lysophospholipids in an aqueous environment. Phospholipids can either surround the cell in a phospholipid bilayer (a) or as a liposome (b). Lysophospholipids have the tendency to form micelles (c) (Jansen, 2015).



Phospholipid Lysophospholipid

Figure 2.4 chemical structure showing the cylindrical phosphatidylcholine and the Lysophosphatidylcholine (Grezelzcyk & Gendaszewska-Darmach, 2013).

In combination with linoleic acid, Lysophosphatidylcholine leads to the formation of smaller and more stable ovalbumin protein emulsions (Mine *et al*., 1993; Jansen, 2015). A smaller and more stable micelle would lead to improved lipid absorption across the unstirred water layer within the GIT of birds. In pigs it was demonstrated that the ileal amino acid digestibility was increased when their diets were supplemented with a lysophospholipid based emulsifier (Van Barneveld *et al.,* 2003). Carter & Henman (2003) also demonstrated improved weaner growth performance, while

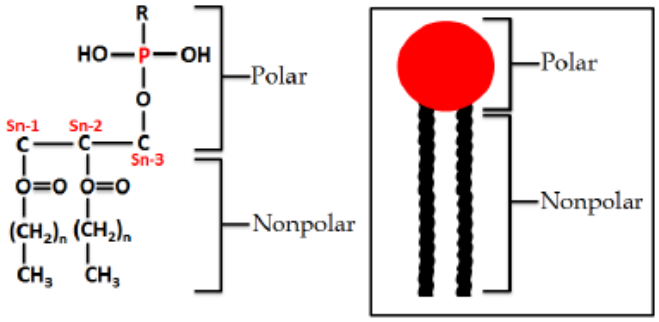
Carter & Perez-Maldonada (2007) reported an improvement in weight gain for broilers when lysophospholipids were added to their diet.

2.4.1 Phospholipids

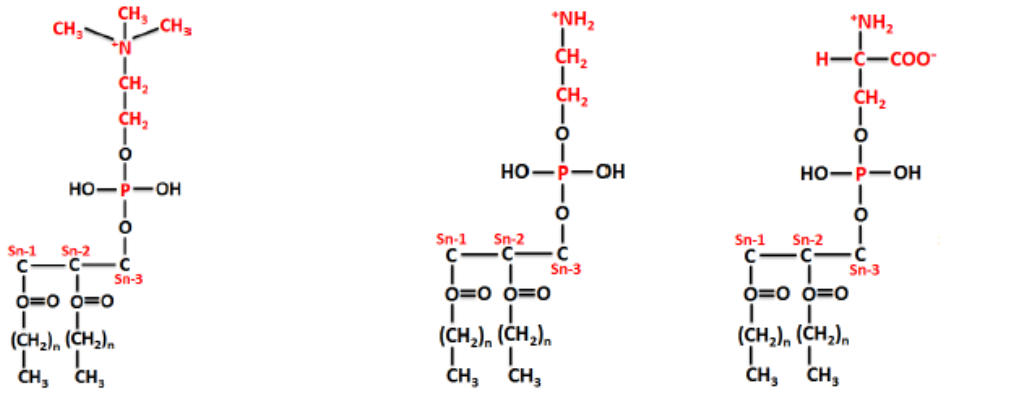
Only a few studies have focused on phospholipids due to them being essential constituents of cellular membranes and being amphipathic (Dowhan, 1997; Vance & Vance, 2002; Vares *et al*., 2003). Phospholipid application extends beyond its use in animal feeds, but also as an emulsifier in pharmaceuticals, food and preparation of liposomes for cosmetics and drug delivery (Gabizon *et al.*, 1997; Uhumwangho & Okor, 2005). Phospholipids are characterized by a glycerol backbone with a linked polar phosphodiester group at the *sn*-3 carbon.

Phospholipids can be divided into three structural regions, as shown in Figure 2.5 (AOCS, 2015):

1. A polar hydrophilic headgroup which resides at the lipid-water interface
2. Interfacial region which is of intermediate polarity
3. Hydrophobic tail region



Phospholipid



Phosphatidylcholine Phosphatidylethanolamine Phosphatidylserine

Figure 2.5 Chemical structure of phospholipids (AOCS, 2015).

2.4.2 Lysophospholipids

Lysophospholipids are a result of enzymatic hydrolysis of phospholipids (Figure 2.6) and are constructed with a monoacylglycerol in either position *sn*-1, 1-lysophospholipids or *sn*-2, 2-lysophospholipids and a phosphate residue in position *sn*-3. Lysophospholipids are found in small quantities within the cellular membranes, they are good emulsifiers and solubilizing agents and are used in foods, cosmetics and pharmaceuticals same as phospholipids (Reblova & Pokorny, 1995; Birgbauer & Chun, 2006; Dennis *et al.,* 2006). Lysophospholipids also play an important role during reproductive physiology, vascular development and nervous system physiology due to their presence and their receptors in various tissues and cell types (Karliner, 2004; Chun, 2005; Parrill, 2008).

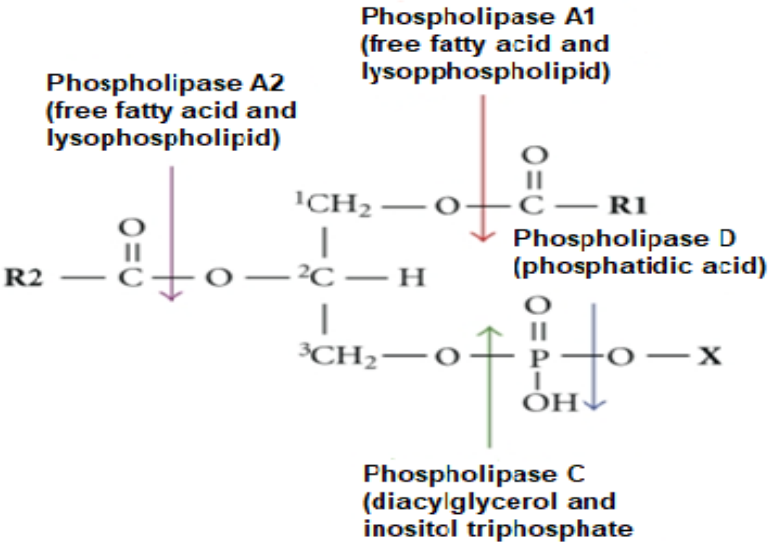


Figure 2.6 Hydrolysis of phospholipids by phospholipases. Arrows indicate the sites of attack for hydrolytic cleavage of phospholipases type A1, A2, C, and D. The main products generated by their action are also shown. R1/R2: Free fatty acids in sn-1 or sn-2 positions; X: Choline, ethanolamine, serine, inositol, and others. (Belaunzaran, *et al.*, 2011)

2.4.3 Mode of action of fat emulsifiers

2.4.3.1 Emulsification and hydrolysis

Phospholipids as well as lysophospholipids have active surface properties due to their hydrophilic head and the hydrophobic tail (FA chains). Lysophospholipids have better oil-in-water emulsifying properties than phospholipids due to the removal of one FA during hydrolysis (Joshi *et al.*, 2006; Liu & Ma, 2011). Lysophospholipids act as an emulsifier in combination with bile salts during the initiation of lipid digestion (Zhang *et al*., 2011). This improved emulsification will result in smaller lipid droplets which creates a bigger interphase area. The bigger interphase area facilitates lipase attachment and improves lipid hydrolysis. Lipase absorption and activity can also be affected by the surface-active compounds, which include phospholipids and lysophospholipids (Dahim & Brockman, 1998; Reis *et al.*, 2008; Mandalari *et al.*, 2009; Reis *et al.*, 2010; Malaki *et al.*, 2011; Maldonado-Valderrama *et al.*, 2011; Verrijsen, 2015). Through the removal of monoglycerides and FFA from the lipid interphase, lipid hydrolysis will be improved, creating another potential mode of action of lysophospholipids (Zhang *et al.,* 2011). Biosurfactants (emulsifiers) are required to remove these products to the aqueous gut lumen and this is done by the formation of mixed micelles through the assistance of lysophospholipids

2.4.3.2 Lipid absorption

Phospholipids and lysophospholipids play an important role in cell membrane structures and in cell signaling. Lysophospholipids also increase the fluidity and permeability of cell membranes (Lundbaek & Andersen, 1994; Wendel, 2000; Lundbaek 2006). Lysophospholipids have a direct or indirect effect on membrane protein formation and function (Lundbaek & Andersen, 1994; Maingret *et al.*, 2000; Lundbaek, 2006), which influences the uptake of lipids across enterocytes in the small intestine. Lysophospholipids incorporate monoglycerides and FFA into mixed micelles; this improves the transportation through the unstirred water layer. By increasing the lysophospholipid content in the lumen, smaller micelles will be formed, and micelle transportation as well as lipid absorption will be improved (Lundbaek, 2006).

2.5 Broiler nutrition

Lipids provide the main source of energy to animals and have the highest caloric value among all nutrients (Zhao & Kim 2017). Lipids form an important component and perform vital functions within the animal's body. The oils and fats of natural resources are incorporated in poultry feed to enhance the energy contents of the diets (Siyal *et al*., 2017). Given the amount of lipids added to broiler diets, the use of exogenous emulsifiers can positively impact on the performance of the birds (Zampiga *et al*., 2016). The mode of action of emulsifiers is to increase the active surface of fats, allowing the action of lipase, which hydrolyse triglyceride molecules into fatty acids and monoglycerides and favour the formation of micelles consisting of lipolysis products. This is an essential step for lipid absorption, as it creates a diffusion gradient that increases absorption (Guerreiro Neto *et al*., 2011). There is literature available showing the effect of fat emulsifiers on the overall production performance of broilers (Nir et al., 1993; Azman & Siftici, 2004; Melegy *et al*., 2010; Zosangpuii *et al*., 2011; Aguilar *et al*., 2013; Zaeferian *et al*., 2011; Boontiam *et al*., 2016., San Tan *et al*., 2016; Zampiga *et al*., 2016; Zavareie & Toghyani, 2018).

2.5.1 Broiler production parameters

The ability to digest lipids is not fully developed in the young chick. Due to this inadequate development, there were no differences observed for average live weight during the first two weeks when vegetable oils were used, while animal fat digestion only improved after 8 weeks (Freeman, 1976; Kroghdahl, 1985). However, in trials by Nir *et al*. (1993) the results reported

showed significant differences at 14 days for live weight when emulsifiers were used on different fat sources. The results for live weight at day 21 by Zobac *et al*. (1998) showed that body weight of birds fed diets containing lecithin increased significantly. Emmert *et al*. (1996) also observed that there was an improvement of body weight of young birds. In contrast to these results, Azman & Siftici (2004) as well as Zavareie & Toghyani (2018), both using lecithin supplements, indicated that the body weights of birds at day 21 were not affected by lecithin supplementation. The reason for their results on the young birds indicated the role which phospholipids play in fat digestion through their emulsification properties as well as nutrient absorption by increasing micelle formation, resulting in improved growth performance in young birds (Schwarzer & Adams, 1996). Both, San Tan *et al*. (2016) and Roy *et al*. (2010), used exogenous emulsifiers and reported significant improvement of body weight gains at day 35. Melegy *et al*. (2010) also confirmed these results where the addition of lysolecithin significantly improved body weight gain. In results obtained by Zampiga *et al*. (2016) using soya oil they observed no significant difference on final body weight with the addition of an emulsifier - indicating that the effect of emulsifiers is less significant on unsaturated fat sources (Jansen 2015).

Siyal *et al*. (2017), Roy *et al*. (2010) and Zosangpuii *et al*. (2011), who all used exogenous emulsifiers, observed a higher feed intake on these treatments. Similarly, Zaeferian *et al*. (2015) who used 3.5 kg/ton lysophospholipid, observed a significant increase in feed consumption. The positive effect on feed intake when an emulsifier is added could be because of improved palatability, which can lead to a higher feed and energy intake (Cho *et al*., 2012). These results were however contradicting Guerreiro *et al*. (2011), Aguilar *et al*. (2013) and Zhang *et al*. (2011) who used casein, a nonionic and lysophosphatidyl-choline emulsifier respectively and who observed no significant effect on feed intake of broilers. Zampigy *et al*. (2016) also observed no significant difference on ADG when using an emulsifier at a constant inclusion of 1 kg/ton. This was however in contrast with Melegy *et al*. (2010) who used lysolecithin at 0.25 and 0.5 kg/ton and showed significantly higher ADG when an emulsifier was added. Addition of an emulsifier on different fat sources had no influence on broiler performance, Ferreira *et al*. (2005) did not observe performance differences among broilers fed different ratios of soybean oil and tallow, while Sanz *et al*. (2000[a]), used sunflower oil and a blend of tallow and lard, and Manilla *et al*. (1999) and Andreotti *et al*. (2004), with various levels of soya oil in the diet. Danicke *et al*. (1997) reported an improved live weight gain and FCR in birds fed soya oil diet (100 g/kg) than in chicks that were fed diets containing tallow. Melegy *et al*. (2010), Siyal*et al*. (2017), Roy *et al*. (2010), Zampiga *et al*. (2016) and Zosangpuii *et al*. (2011), all observed improved FCR when exogenous emulsifiers were used. In contrast to these results, Guerreeiro Neto *et al*. (2011) observed no significant difference in feed conversion ratio with the addition of an emulsifier to the diet of broilers. This

contradicting literature could be due to the FA composition of the fat sources used in the individual trials; the utilization of dietary fat in broiler diets increases when the ratio between unsaturated and saturated FA increases from 0.0 to 2.5 (Ketels & DeGrootte, 1989).

2.5.2 Broiler carcass parameters

Roy *et al*. (2010), Zampiga *et al*. (2016), Guerreiro Neto *et al*. (2011) and Aquilar *et al*. (2013), using various fat emulsifiers in the diets showed that the addition of the emulsifier had no effect on the dressing percentage of broilers. Cho *et al*. (2012) and Zavareie & Toghyani (2018), who used sodium steroyl-2-lactylate and a phospholipid, respectively, also observed no difference on carcass dressing percentage. Contradictory to these results, Melegy *et al*. (2010), showed a significant increase of dressing percentage when birds were supplemented with Lysoforte Booster in comparison to the control group. The reason for these contradictory results may be due to the fat sources used in the various experiments. The utilization of an unsaturated lipid reduces fat and results in an increase in carcass protein resulting in an increased dressing percentage as the energy derived from unsaturated fat may be used for other metabolic purposes, while energy derived from saturated fat sources is not well utilized and thus accumulates as body fat resulting in a decreased dressing percentage (Sanz *et al*., 2000[b]). Pigs that were fed lysophospholipids, were found to have similar slaughter yields with or without an emulsifier in their diet (Schwarzer & Adams 1996).

Melegy *et al*. (2010), observed no significant difference between breast and thigh weight when Lysoforte Booster emulsifier additive was used. Also, Andreotti *et al*. (2004), Ferreira *et al*. (2005), Lara *et al*. (2006), Guerreiro Neto *et al*. (2011), Aquilar *et al*. (2013) and Zampiga *et al*. (2016) reported no significant difference in carcass portions when different fat sources or emulsifier were used in broiler diets. In contrast, Boontiam *et al*. (2016), showed the leg weights were heavier in the diets without an emulsifier but not significantly heavier than the leg weights in the control diet.

2.5.3 Broiler organ characteristics

Cho *et al*. 2012, Abbas *et al*. (2016), Andreotti *et al*. (2004), Roy *et al*. (2010), Luc *et al*. (2013), Ferreira *et al*. (2005), Lara *et al*. (2006) and Guerreeiro Neto *et al*. (2011), reported that when an emulsifier was added to the diet, no significant difference were observed on relative organ weights of the chickens. Contradictory to these results, Praharaj *et al*. (1997), showed a significant difference in the internal organ weights when an emulsifier was used in the diet of broiler chicks.

These results coincide with the results obtained by Siyal *et al*. (2017), Huang *et al*. (2007) and Nagargoje *et al*. (2016) who all observed the liver to have a higher weight when adding a soy lecithin into the diet. Lipid metabolism occurs predominantly in the liver and up to 95% of *de novo* fatty acid synthesis occurs here (Theil & Lauridsen, 2007), therefore the increased liver weight could indicate increased lipid metabolism.

However, Boontiam *et al*. (2016) reported that the addition of an emulsifier had no significant effect on the immune organ (spleen, thymus and bursa of Fabricius) weights. Similarly, Andreotti *et al*. (2004), Ferreira *et al*. (2005), Lara *et al*. (2006), Roy *et al*. (2010), Guerreeiro Neto *et al*. (2011), Cho *et al*. (2012), Wang *et al*. (2016) and Siyal *et al*. (2017) also observed no significant differences on lymphoid organs when various emulsifiers were used.

2.6 Conclusion

Emulsifiers have been tested under a variety of conditions and on many different fat sources in broiler chickens. Production performance parameters for body weight gain, feed intake and feed conversion ratio were all improved significantly when emulsifiers were added to the diets. Although there were some contradicting results obtained, the main factor impacting the results was the type of fat used in the trails as the effect of emulsifiers is less significant on unsaturated fat sources. On carcass characteristics, most of the results showed no significant difference on carcass dressing percentage or portion yield. Similarly, on the organ characteristics, there were also no significant differences between the studies which shows there is no adverse effect from the use of emulsifiers on broilers.

The emphasis of this trial was to use two commercially available oils, namely refined soya oil and a lower quality unsaturated blend of animal fats and vegetable oils. The dietary energy was reduced, and the aim is for the emulsifier to improve fat utilization to overcome the dietary energy deficit with no impact on broiler performance, carcass and organ characteristics.

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Chapter 3

The effect of two different oil sources and addition of a lysophospholipid on production parameters of broiler chickens

3.1 Abstract

The study investigated the effect of two different oils and for each type of oil, three diets were formulated, the first with standard AME and the other two containing 0.25 MJ/kg less energy. One of the reduced diets included a lysophospholipid - Lysoforte® Extend Dry (LEX) - at an inclusion level of 500 g/ton. The two oils used was refined soya oil and a lower quality unsaturated blend of animal fats and vegetable oil. Two thousand, one hundred and twelve chicks were randomly allocated to six treatments, where each treatment was replicated sixteen times. The broiler chickens were raised until slaughter at day 35 of age. No significant differences were observed for any parameter measured except for day 35 average live weight between the two decreased AME diets with soya oil and blended oil (CONS- and CONBO-) as well as the blended oil treatment with LEX (CONBO+). The soya oil diets (CONS, CONS- and CONS+) at day 35 was significantly heavier than the CONBO- and CONBO+. Similarly, the average live weight between the groups including LEX at day 21 and day 28 were significantly higher for the soya oil treatment compared to the blended oil treatment. It can therefore be concluded that the dietary energy can be reduced when using a good quality oil, such as soya oil, by including LEX to the diet with no effect on growth performance of broilers.

Keywords– Poultry, performance, ADG, FCR, PER, EPEF, AME, lysophospholipid

3.2 Introduction

In broiler nutrition, lipids, which refers to animal fats and vegetable oils, are used to obtain energy dense diets required by the modern broiler for optimal growth performance thereby achieving the industry standards (Blanch *et al*., 1996). Genetics has resulted in birds that grow faster with improved feed conversion ratio (FCR) which highlights the importance of the birds to utilize all the macro ingredients of the energy dense feed (Svihus, 2014; Cherian, 2015). Fats not only have the highest caloric value of all macro ingredients used but, the net energy obtained from metabolizable energy is 90%, showing the value of fats in broiler diets (Scott *et al*., 1983, Lehninger, *et al*., 2008). Mouro (2003) showed that on diets containing oil, the birds had a better performance than birds receiving a diet with no oil. Besides energy supply, the addition of fats to

the diet also improves the absorption of fat-soluble vitamins, diminishes the purveyance, increases the palatability of the feed, increases the efficiency of the consumed energy and reduces the passage rate of digestion through the gastrointestinal tract allowing for an improved absorption of all other nutrients that are present in the diet (Baiao & Lara, 2005).

Metabolic transformation is required to convert carbohydrates and proteins to fatty acids which produces heat, while adequate fat levels in the diet will prevent this process from occurring and result in a reduced heat increment (Murugesan, 2013). During hot weather conditions, it is frequently recommended to compensate for a decreased feed intake through the increase of protein and energy levels in the feed. Fat presents the lowest heat increment (9%) compared to protein (26%), which is the highest and then carbohydrates (15%). Thus, when the proportion of energy from fat is increased, the bird would be able to handle heat stress more easily (Ribeiro & Lagana, 2002). Dale & Fuller (1980) observed that, when birds were exposed to cyclic heat stress, growth rate improved when fat was added to the diet. However, fat had no effect when birds were submitted to chronic heat stress. The reason for this being that birds can dissipate heat during the cooler night period, which is not possible when birds are exposed to constant high temperatures.

There are several factors that could influence lipid digestion in birds which could be either animal characteristics or diet related factors. Animal characteristics can be factors such as age of the bird, where the digestibility is lower in younger animals as lipid metabolism is not yet fully developed in young birds (Krogdahl, 1985; Wiseman, 1990; Baiao & Lara, 2005; Tancharoenrat *et al*., 2013). Bile salts is the first limiting factor followed by lipase secretion (Krogdahl, 1985; Ketels, 1994). The lowered lipid utilization in young birds is attributed to low bile salt concentration (Krogdahl, 1985; Meng *et al*., 2005). Gender and genetic strain also have an impact on lipid digestibility and was observed to be higher in female broilers by Guirguis (1975), while Slinger *et al*. (1955) found that male broilers had a better growth performance over female broilers due to their superior ability for lipid digestion. Similarly, it was observed that male broilers have a higher growth rate and feed efficiency while female broilers tend to have a higher fat deposition rate (Becker *et al.*, 1981; Shalev & Pasternak, 1998; Huang *et al.*, 2008; Abdullah *et al.*, 2010). Dietary lipids can alter the microbial community of broilers (Knarreborg *et al*., 2002; Yang *et al*., 2009; Van der Hoeven-Hangoor *et al*., 2013) which will affect lipid digestibility. The microbiota is involved in the conversion of primary bile salts into secondary bile salts through the process of microbial deconjugation and dihydroxylation. This results in a more hydrophobic bile salt that will decrease the bile salts effectiveness of lipid digestion (Krogdahl, 1985; Drackley, 2000).

Diet related factors include degree of saturation and chain length of fatty acids. Lipid sources used in broiler diets are not all utilized equally by the bird due to the differences in fatty acid profile, unsaturated/saturated ratio and oxidative state (Freeman, 1984; Krogdahl, 1985). Saturated fatty acids, especially long chain FA have a lower digestibility and absorption rate in broilers when compared to short chain FA, medium chain FA and unsaturated FA. Increasing lipid inclusion levels in poultry diets lead to a decreased lipid digestibility, this is due to the limited availability of lipase and bile salts for the increasing amounts of lipid. This is more pronounced in young broilers (Krogdahl, 1985; Wiseman *et. al.*, 1991; Blanch *et al*., 1996; Sanz *et al.*, 2000; Villaverde *et al.*, 2006; Smink, 2012). Dietary calcium level and type of fatty acid impacts calcium metabolism and soap formation. The hydrolysis of triacylglycerides forms monoglycerides and FFA; these FFA can react with other nutrients to form soluble and insoluble soaps. Insoluble soaps cause the FA and the mineral that it's bound to, to be unavailable to the animal (Leeson & Summers, 2005). Tancharoenrat & Ravindran (2014) identified calcium-phytate as a substrate during the formation of insoluble metallic soaps in the gastrointestinal tract of broilers.

Lipids are water insoluble compounds and the digestion takes place in an aqueous environment in the small intestines through the synergistic action of bile salts and pancreatic lipase. Bile salts ensure the emulsification of dietary fats which allows pancreatic lipase to hydrolyze the triglycerides that are present on the water-oil interface. This produces 2-monoglycerides and free fatty acids (Leeson & Summers, 2001; Zampiga *et al*., 2016). Bile salts play a major role in mixed micelle formation which are absorbed on the mucosa cells in the small intestines (Kroghdahl, 1985). Where lipids are added to broiler diets, the use of an exogenous emulsifier can improve the emulsion and micelle formation. This leads to an improved lipid digestion and productive performance (Jansen *et al*., 2015; Zampiga *et al*., 2016). Lysophospholipids are formed through the hydrolysis of the ester bond of phospholipids. This process results in improved emulsification of fat into smaller droplets which has a larger surface area for lipase enzymes to work on. Lysophospholipids have a lower critical micelle concentration and form smaller micelles when compared to phospholipids (Reynier *et al*., 1985; Zubay, 1983; Zampiga *et al*., 2016). Lysophospholipids are important in animal nutrition as biosurfactants and with the lipophilic and hydrophilic properties they contain, helps with their role as biosurfactants when they are mixed with water and lipids. The addition of lysophospholipids to the diet showes an increased absorption and digestion of fat in the young chick (Sugumar, 2012). The effectiveness of emulsifiers is dependent on the composition of the supplemental fat which include chain length, position of FA, degree of saturation and the level of dietary fat (Dierick & Decuypere, 2004; Jansen, 2015). The effect of emulsifiers on fat digestion is less in unsaturated fat sources than with saturated fat sources (Huyghebaert, 2003; Jansen 2015).

Bindhu *et al*. (2011) noted that emulsifiers could be used to partially replace dietary fat, without influencing the performance of the broilers. In another study which was conducted on low nutrient density diets by Melegy *et al*. (2010), it was demonstrated that lysophospholipids could be used to compensate for these low-density diets without affecting the birds' performance. Bindhu *et al*. (2011) compared three different fat sources, namely: palm oil, rice bran oil and tallow in diets with a synthetic emulsifier inclusion of 125 ppm. This was used to compare the energy sparing effect of emulsifiers through the increase in apparent metabolizable energy levels of the different fat sources. The best results were obtained in the palm oil diet where the apparent metabolizable energy was increased by 0.41 MJ/kg, while rice bran oil and tallow had an improvement of 0.36 MJ/kg and 0.34 MJ/kg, respectively. This highlights the importance of adding emulsifiers to low metabolizable energy diets, which resulted in a potential cost saving. Where lysophospholipids were added to diets containing pig lard, there was a significant improvement in fat digestion. However, when lysophospholipids were added to diets containing soya oil, only a slight improvement was observed, showing the effect emulsifiers have are dependent on the quality and degree of saturation of the fat sources (Huyghebaert, 2003; Zampiga *et al*., 2016).

Guerreiro *et al*. (2011) observed no difference in performance when different fat sources were fed with additional emulsifiers, which was also the same result observed by Nir *et al*. (1993) who concluded that the seven-day performance of broilers were not improved through the addition of emulsifiers. On the 14-day weight there was a significant effect on the body weight gain and feed conversion ratio between soya oil and poultry fat, and the addition of an emulsifier to each of these (Guerreiro *et al*., 2011). Abbas *et al*. (2016) observed that there was no significant interaction between fat and emulsifiers on feed intake during starter, grower and finisher phases of broilers. On the feed conversion ratio of starter and grower phases, no significant differences were observed, but there was a difference on the finisher phase with the inclusion of an emulsifier in the diet. Zampiga *et al*. (2016) observed the same trend as the birds had a significantly lower feed conversion ratio when synthetic emulsifiers were added. According to Roy *et al*. (2010), emulsifiers should not be added in the starter phases as lipase activity is still low in the young chick. They also observed that fat digestion and absorption was improved when emulsifiers were added to the grower diet (Roy *et al*., 2010).

The aim of this trail was to investigate the effect of two oils and the inclusion of a lysophospholipid on the production parameters of broiler chickens.

3.3 Materials and Methods

3.3.1 Oil components

Two types of oils were used for this trial:

1. Refined soya oil (sourced form Majesty oils, 6 Mould Street, Boltonia, Krugersdorp, Gauteng).
2. Blended oil – an unsaturated blend of animal fats and vegetable oil with a maximum FFA content of 10% (sourced from Energy oils, 165 Tedstone Road, Wadeville, Gauteng)

Representative samples of both oils were analysed at Chem Nutri Analytical Lab (4 Porcelain Road, Clayville, Johannesburg, Gauteng) for free fatty acids (FFA) (AOAC Ca 5a-40), total saturated fatty acids (SFA) (AOAC 977.17), total unsaturated fatty acids (UFA) (AOAC 977.17) and moisture (AOCS Ca 2c-25), impurities (AOCS Ca 3a-46) and unsaponifiables (AOCS Cs 6b-53) (MIU). From these results, the Wiseman equation (Wiseman, 1999) was used to calculate the AME for 0 to 21-day broilers as well as > 21-day broilers. The equation used is:

Equation 1

$$AME\ (MJ/kg\ fat) = A + B \times FFA + C \times e^{(D \times U/S)}$$

Where:

AME = Apparent Metabolizable Energy

FFA = Free fatty acids

U/S = Unsaturated fatty acids / Saturated fatty acids ratio

A, B, C and D constant values are shown in Table 3. below

Table 3.1 Constants A, B, C, and D in the AME prediction equation (Wiseman, 1999)

Constants values	Young birds 0-21 days	Older Birds > 21 days
A	38.112	39.050
B	-0.009	-0.006
C	-15.337	-8.505
D	-0.509	-0.403

3.3.2 Experimental diets

The chicks were assigned to six different treatment diets, the diet specifications are shown in Table 3.22. Ingredient and calculated nutrient composition of the treatment diets are shown in Table 3.3, Table 3.4 and Table 3.5. Format® formulation software was used for all the formulations using the least cost optimization function that optimizes each formulation to the pre-set specifications. The nutrient specifications used were within the prescribed minimum specifications by Ross 308 (Aviagen group, 2014[b]). Samples of the two oils were sent to Kemin Industries Incorporated for a lipid evaluation test, lab batch number CS_SSA 17050051. The results obtained are shown in Table 3.6. For soya oil and the blended oil an AME of 36.69 MJ/kg and 30.78 MJ/kg were used, respectively as obtained from the lipid evaluation test. Mixing of the experimental feed occurred at Wisium SA (Pty) LTD feed plant (3 Piet Rautenbach Street, Brits, North West) where small batches were mixed, and all feeds were pelletized. A Buhler pellet press was used, running at temperature of 72 ℃, producing pellets of 3.5mm in diameter. The starter diets were crumbled after being pelletized. The starter feed was fed from day 0 until day 14, the grower phase was fed from day 14 until day 28 and finisher feed was fed from day 28 until slaughter, at day 35. Diets 1 (CONS) and 4 (CONBO) were the control diets for soya oil and the blended oil, respectively, diets 2 (CONS-) and 3 (CONS+) with soya oil and diets 5 (CONBO-) and 6 (CONBO+) with the blended oil had a reduced energy value of 0.25 MJ/kg. The lysophospholipid, LEX, was added to CONS+ and CONBO+ respectively at an inclusion level of 500 g/ton in the finished feed through all the phases.

Table 3.2 Trial specifications for all treatments and phases as per the breed nutrient specifications on the control diets (Aviagen group, 2014[b])

Parameter	Unit	CONS[2]	CONS-[3]	CONS+[4]	CONBO[5]	CONBO-[6]	CONBO+[7]
Starter (0-14 days)			**Soya oil**			**Blended oil**	
AME[1]	**MJ/kg**	11.15	10.90	10.90	11.15	10.90	10.90
Crude fat (minimum value)	**%**	3.50	3.50	3.50	3.50	3.50	3.50
Available phosphorous	**%**	0.50	0.50	0.50	0.50	0.50	0.50
Calcium	**%**	1.05	1.05	1.05	1.05	1.05	1.05
Digestible Lysine	**%**	1.20	1.20	1.20	1.20	1.20	1.20
Grower (14-28 days)							
AME[1]	**MJ/kg**	11.60	11.35	11.35	11.60	11.35	11.35
Crude fat (minimum value)	**%**	3.50	3.50	3.50	3.50	3.50	3.50
Available phosphorous	**%**	0.40	0.40	0.40	0.40	0.40	0.40
Calcium	**%**	0.84	0.84	0.84	0.84	0.84	0.84
Digestible Lysine	**%**	1.10	1.10	1.10	1.10	1.10	1.10
Finisher (28-35 days)							
AME[1]	**MJ/kg**	11.80	11.55	11.55	11.80	11.55	11.55
Crude fat (minimum value)	**%**	3.50	3.50	3.50	3.50	3.50	3.50
Available phosphorous	**%**	0.36	0.36	0.36	0.36	0.36	0.36
Calcium	**%**	0.76	0.76	0.76	0.76	0.76	0.76
Digestible Lysine	**%**	1.05	1.05	1.05	1.05	1.05	1.05

([1])Apparent Metabolizable Energy
([2]) Control diet with soya oil, ([3]) Decreased AME of 0.25 MJ/kg with soya oil ([4]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX ([5]) Control diet with blended oil ([6]) Decreased AME of 0.25 MJ/kg with blended oil ([7]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

Table 3.3 Ingredients and calculated nutrient composition of the trial starter diets

Ingredients	Unit	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
Yellow Maize	%	55.070	56.470	56.470	54.400	56.170	56.170
Soya Oilcake	%	33.430	33.200	33.200	33.570	33.270	33.270
Sunflower Oilcake	%	4.370	4.370	4.370	4.370	4.370	4.370
Feedlime	%	1.670	1.680	1.680	1.670	1.680	1.680
MCP[a]	%	1.160	1.160	1.160	1.160	1.160	1.160
Soya Oil	%	2.160	1.000	1.000	-	-	-
Gluten 60	%	0.670	0.670	0.670	0.670	0.670	0.670
Sodium Bicarbonate	%	0.360	0.370	0.370	0.360	0.370	0.370
Lysine	%	0.290	0.300	0.300	0.290	0.290	0.290
Methionine	%	0.290	0.290	0.290	0.290	0.290	0.290
Broiler Starter Premix	%	0.150	0.150	0.150	0.150	0.150	0.150
Salt	%	0.130	0.130	0.130	0.130	0.130	0.130
Choline chloride	%	0.100	0.100	0.100	0.100	0.100	0.100
Threonine	%	0.060	0.060	0.060	0.060	0.060	0.060
Avatec[c]	%	0.050	0.050	0.050	0.050	0.050	0.050
Axtra PHY 10000P[b]	%	0.010	0.010	0.010	0.010	0.010	0.010
Stafac 500[d]	%	0.004	0.004	0.004	0.004	0.004	0.004
Blended oil	%	-	-	-	2.700	1.270	1.270
Lysoforte Extend Dry	%	-	-	0.050	-	-	0.050
Calculated Nutritional Value							
Moisture	%	10.860	11.000	11.000	10.790	10.970	10.970
AME chick	MJ/kg	11.150	10.900	10.900	11.150	10.900	10.900
Crude protein	%	22.060	22.070	22.070	22.070	22.060	22.060
Crude fat	%	4.740	3.630	3.630	5.200	3.860	3.860
Crude fibre	%	4.540	4.570	4.570	4.520	4.560	4.560
Ash	%	5.940	5.940	5.940	5.940	5.940	5.940
Linoleic Acid	%	1.320	1.350	1.350	1.310	1.340	1.340
Calcium	%	1.050	1.050	1.050	1.050	1.050	1.050
Phosphorous	%	0.660	0.660	0.660	0.660	0.660	0.660
Available phosphorous	%	0.500	0.500	0.500	0.500	0.500	0.500
Sodium	%	0.160	0.160	0.160	0.160	0.160	0.160
Chloride	%	0.200	0.200	0.200	0.200	0.200	0.200
Potassium	%	0.900	0.900	0.900	0.900	0.900	0.900
Digestible Lysine	%	1.200	1.200	1.200	1.200	1.200	1.200
Digestible Methionine	%	0.590	0.590	0.590	0.600	0.590	0.590
Digestible Methionine + Cystine	%	0.890	0.890	0.890	0.890	0.890	0.890
Digestible Threonine	%	0.760	0.760	0.760	0.760	0.760	0.760
Digestible Tryptophan	%	0.220	0.220	0.220	0.220	0.220	0.220
Digestible Isoleucine	%	0.820	0.820	0.820	0.820	0.820	0.820
Digestible Arginine	%	1.320	1.320	1.320	1.320	1.320	1.320
Digestible Histidine	%	0.510	0.510	0.510	0.510	0.510	0.510
Digestible Leucine	%	1.680	1.690	1.690	1.680	1.690	1.690
Digestible Valine	%	0.900	0.900	0.900	0.900	0.900	0.900
Digestible Glycine + Serine	%	1.680	1.680	1.680	1.680	1.680	1.680

[a]Mono Calcium Phosphate

[b]Phytase enzyme used

[c]Anticoccidial used (Lasalocid sodium)

[d]Antibiotic growth promoter (Virginiamycin)

([1]) Control diet with soya oil, ([2]) Decreased AME of 0.25 MJ/kg with soya oil ([3]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX ([4]) Control diet with blended oil ([5]) Decreased AME of 0.25 MJ/kg with blended oil ([6]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

Table 3.4 Ingredients and calculated nutrient composition of the trial grower diets

Ingredients	Unit	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
Yellow Maize	%	61.500	62.870	62.870	60.900	62.630	62.630
Soya Oilcake	%	26.400	26.130	26.130	26.500	26.200	26.200
Sunflower Oilcake	%	4.000	4.000	4.000	4.000	4.000	4.000
Gluten 60	%	2.700	2.700	2.700	2.700	2.700	2.700
Feedlime	%	1.400	1.400	1.400	1.400	1.400	1.400
Soya Oil	%	1.920	0.770	0.770	-	-	-
MCP[a]	%	0.630	0.620	0.620	0.630	0.620	0.620
Sodium Bicarbonate	%	0.400	0.400	0.400	0.400	0.400	0.400
Lysine	%	0.360	0.360	0.360	0.360	0.360	0.360
Methionine	%	0.250	0.250	0.250	0.250	0.250	0.250
Broiler Grower Premix	%	0.150	0.150	0.150	0.150	0.150	0.150
Salt	%	0.110	0.100	0.100	0.110	0.100	0.100
Choline chloride	%	0.100	0.100	0.100	0.100	0.100	0.100
Threonine	%	0.060	0.060	0.060	0.060	0.060	0.060
Avatec[c]	%	0.050	0.050	0.050	0.050	0.050	0.050
Axtra PHY 10000P[b]	%	0.010	0.010	0.010	0.010	0.010	0.010
Stafac 500[d]	%	0.004	0.004	0.004	0.004	0.004	0.004
Blended oil	%	-	-	-	2.400	0.970	0.970
Lysoforte Extend Dry	%	-	-	0.050	-	-	0.050
Calculated Nutritional Value							
Moisture	%	11.090	11.240	11.240	11.030	11.210	11.210
AMEn chick	MJ/kg	11.600	11.350	11.350	11.600	11.350	11.350
Crude protein	%	20.580	20.580	20.580	20.590	20.590	20.590
Crude fat	%	4.630	3.530	3.530	5.030	3.690	3.690
Crude fibre	%	4.310	4.340	4.340	4.300	4.340	4.340
Ash	%	4.800	4.810	4.810	4.800	4.800	4.800
Linoleic Acid	%	1.440	1.470	1.470	1.430	1.460	1.460
Calcium	%	0.840	0.840	0.840	0.840	0.840	0.840
Phosphorous	%	0.520	0.530	0.530	0.5200	0.520	0.520
Available phosphorous	%	0.400	0.400	0.400	0.400	0.400	0.400
Sodium	%	0.160	0.160	0.160	0.160	0.160	0.160
Chloride	%	0.200	0.200	0.200	0.200	0.200	0.200
Potassium	%	0.780	0.770	0.770	0.780	0.770	0.770
Digestible Lysine	%	1.100	1.100	1.100	1.100	1.100	1.100
Digestible Methionine	%	0.560	0.550	0.550	0.560	0.550	0.550
Digestible Methionine + Cystine	%	0.840	0.840	0.840	0.840	0.840	0.840
Digestible Threonine	%	0.690	0.690	0.690	0.690	0.690	0.690
Digestible Tryptophan	%	0.190	0.190	0.190	0.190	0.190	0.190
Digestible Isoleucine	%	0.750	0.750	0.750	0.750	0.750	0.750
Digestible Arginine	%	1.160	1.150	1.150	1.160	1.160	1.160
Digestible Histidine	%	0.470	0.470	0.470	0.470	0.470	0.470
Digestible Leucine	%	1.710	1.720	1.720	1.710	1.720	1.720
Digestible Valine	%	0.830	0.830	0.830	0.830	0.830	0.830
Digestible Glycine + Serine	%	1.540	1.540	1.540	1.540	1.540	1.540

[a]Mono Calcium Phosphate

[b]Phytase enzyme used

[c]Anticoccidial used (Lasalocid sodium)

[d]Antibiotic growth promoter (Virginiamycin)

([1]) Control diet with soya oil, ([2]) Decreased AME of 0.25 MJ/kg with soya oil ([3]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX ([4]) Control diet with blended oil ([5]) Decreased AME of 0.25 MJ/kg with blended oil ([6]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

Table 3.5 Ingredients and calculated nutrient composition of the trial finisher diets

Ingredients	Unit	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
Yellow Maize	%	63.370	64.770	64.770	62.630	64.400	64.400
Soya Oilcake	%	25.270	25.030	25.030	25.400	25.070	25.070
Sunflower Oilcake	%	4.000	4.000	4.000	4.000	4.000	4.000
Soya Oil	%	2.330	1.170	1.170	-	-	-
Gluten 60	%	1.970	1.970	1.970	1.970	1.970	1.970
Feedlime	%	1.280	1.280	1.280	1.280	1.280	1.280
MCP[a]	%	0.400	0.390	0.390	0.400	0.390	0.390
Sodium Bicarbonate	%	0.390	0.390	0.390	0.390	0.390	0.390
Lysine	%	0.330	0.340	0.340	0.330	0.340	0.340
Methionine	%	0.240	0.240	0.240	0.240	0.240	0.240
Salt	%	0.110	0.110	0.110	0.110	0.110	0.110
Choline chloride	%	0.100	0.100	0.100	0.100	0.100	0.100
Broiler Finisher Premix	%	0.100	0.100	0.100	0.100	0.100	0.100
Threonine	%	0.050	0.050	0.050	0.050	0.050	0.050
Avatec[c]	%	0.050	0.050	0.050	0.050	0.050	0.050
Axtra PHY 10000P[b]	%	0.010	0.010	0.010	0.010	0.010	0.010
Stafac 500[d]	%	0.004	0.004	0.004	0.004	0.004	0.004
Blended oil	%	-	-	-	2.930	1.470	1.470
Lysoforte Extend Dry	%	-	-	0.050	-	-	0.050
Calculated Nutritional Value							
Moisture	%	11.160	11.310	11.310	11.090	11.270	11.270
AMEn chick	MJ/kg	11.800	11.550	11.550	11.800	11.550	11.550
Crude protein	%	19.700	19.710	19.710	19.700	19.700	19.700
Crude fat	%	5.040	3.930	3.930	5.550	4.180	4.180
Crude fibre	%	4.310	4.340	4.340	4.290	4.330	4.330
Ash	%	4.420	4.420	4.420	4.420	4.420	4.420
Linoleic Acid	%	1.450	1.480	1.480	1.440	1.470	1.470
Calcium	%	0.760	0.760	0.760	0.760	0.760	0.760
Phosphorous	%	0.470	0.470	0.470	0.470	0.470	0.470
Available phosphorous	%	0.360	0.360	0.360	0.360	0.360	0.360
Sodium	%	0.160	0.160	0.160	0.160	0.160	0.160
Chloride	%	0.200	0.200	0.200	0.200	0.200	0.200
Potassium	%	0.760	0.760	0.760	0.760	0.760	0.760
Digestible Lysine	%	1.050	1.050	1.050	1.050	1.050	1.050
Digestible Methionine	%	0.530	0.530	0.530	0.530	0.530	0.530
Digestible Methionine + Cystine	%	0.800	0.800	0.800	0.800	0.800	0.800
Digestible Threonine	%	0.660	0.660	0.660	0.660	0.660	0.660
Digestible Tryptophan	%	0.180	0.180	0.180	0.180	0.180	0.180
Digestible Isoleucine	%	0.720	0.720	0.720	0.720	0.720	0.720
Digestible Arginine	%	1.120	1.110	1.110	1.120	1.110	1.110
Digestible Histidine	%	0.450	0.450	0.450	0.450	0.450	0.450
Digestible Leucine	%	1.620	1.630	1.630	1.620	1.630	1.630
Digestible Valine	%	0.800	0.800	0.800	0.800	0.800	0.800
Digestible Glycine + Serine	%	1.480	1.480	1.480	1.480	1.470	1.470

[a]Mono Calcium Phosphate

[b]Phytase enzyme used

[c]Anticoccidial used (Lasalocid sodium)

[d]Antibiotic growth promoter (Virginiamycin)

([1]) Control diet with soya oil, ([2]) Decreased AME of 0.25 MJ/kg with soya oil ([3]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX ([4]) Control diet with blended oil ([5]) Decreased AME of 0.25 MJ/kg with blended oil ([6]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

3.3.3 Animals and housing system

The trial was conducted at Sovereign Foods Industries (Pty) Ltd Wincanton commercial broiler farm situated outside Uitenhage, Eastern Cape (33° 48' 10.7" S; 25° 20' 25.5" E). The specific farm consisted of 12 broiler houses of which one was used to conduct the trials. Prior to arrival of the experimental chicks, all houses were washed and disinfected, this farm works on the principal of all in all out, which means that all houses were placed within one week and slaughtering of all houses occur in one week as well. Prior to arrival, all 2112 Ross 308 chicks were sexed at the hatchery and only males were used in the trial. The hatchery supplying the chicks is part of Sovereign foods, situated within 12 km of where the trial took place and all the chicks were from the same parental group. A completely randomized block design was used to allocate the chicks to 96 pens set up in the house, with each pen containing 22 chicks. Six treatments with 16 replicates were used for this trial.

The house was temperature controlled, using a SKOV temperature control system and preheating of the house was done to have the temperature at 35 ℃ upon arrival of the chicks. The systems allow for ventilation which helps to remove the ammonia and carbon dioxide, and evenly distributed oxygen throughout the house. Environmental temperature and lighting within the houses were according to the Ross 308 standard (Aviagen group, 2014[a]). Water and feed were supplied *ad libitum.* Plasson™ nipple drinker line ran through each of the pens, feed was supplied in ring feeders and scratch pans were available for the first 5 days. For the duration of the trial there were two workers that made sure all feeders were filled, the water line had no problem and mortalities were removed, counted and weights recorded on a daily basis.

3.3.4 Performance measurements

Feed consumption was recorded on a pen basis at weekly intervals until slaughter at 35 days of age. Individual feed intake was calculated as an average of the pens after correcting for mortality. Body weight of all birds in a pen was measured at placement and weekly thereafter until slaughter at 35 days of age. All the birds in each pen were weighed together and the average weight for an individual bird in each pen was calculated after correction for mortalities. The measurements of body weight and feed left over were used for calculating average live weight, weekly feed intake, cumulative feed intake, feed conversion ratio (FCR) (Equation 2), protein efficiency ratio (PER) (Equation 3) and European production efficiency factor (EPEF) (Equation 4). Average daily gain was estimated by fitting a linear model to the live weight data with the slope representing the rate

of change and therefore average daily gain (ADG). The formulae used to calculate these production parameters were:

Equation 2

$$\text{Feed conversion ratio} = \frac{\text{Cumulative feed intake (g)}}{\text{Average live weight per chick (g)}}$$

Equation 3

$$\text{Protein efficiency ratio} = \frac{\text{Body weight gain (g)}}{\text{Crude protein intake (g)}}$$

Equation 4

$$\text{European production efficacy factor} = \frac{\text{Liveability \% } \times \text{ Live weight (g)}}{\text{Age (days)} \times \text{Feed conversion ratio}} \times \frac{100}{1}$$

3.3.5 Analytical methodologies

Chemical analyses were performed at the Department of Animal Sciences, Stellenbosch University.

3.3.5.1 Sampling Procedure

Random samples of feed were collected from each treatment and for each phase of the feeding program. Samples were taken at the beginning of each phase from the bags of feed set out during the start of the trial.

3.3.5.2 Dry matter determination

The dry matter (DM) content of the samples was determined according to the Association of Official Analytical Chemists International (AOAC) (2002), official method 934.01. The samples were dried at 100 °C for 24 h. The calculations to calculate moisture and dry matter are shown below in Equation 5.

Equation 5

$$\% \text{ Moisture} = \frac{(A + B) - C}{B} \times \frac{100}{1}$$

$$\% \text{ Dry Matter} = 100 - \% \text{ Moisture}$$

Where:

A = Weight of empty and dry crucible

B = Weight of air dried test sample

C = Weight of crucible and moisture free test sample

3.3.5.3 Crude protein determination

The crude protein content of the feed was determined by measuring the total nitrogen content using a LECO FP528 machine, according to the Dumas combustion method 992.15 described by AOAC (2002). The nitrogen content was directly measured and used to calculate the crude protein content using a factor of 6.25.

3.3.5.4 Crude fat determination

The crude fat content of each treatment feed were determined using the acid hydrolysis fat extraction method using diethyl ether, petroleum ether, ethanol and hydrochloric acid 38% reagent as described by the AOAC (2002), official method 920.39.

3.3.5.5 Ash determination

The duplicate samples used in the dry matter determination (3.2.4.1) were retained and used to analyse the ash content of the feed (AOAC, 2002; official method 942.05). The samples were combusted in a furnace oven at 500 °C for 6 h.

3.3.5.6 Crude fibre determination

The crude fibre in the feed and faeces samples was analysed according to the official method 962.09 (AOAC, 2002) on a Fibertec/Dosifiber extrusion apparatus. The samples were dried in a

100 °C oven for 48 h and then combusted at 500 °C for 6 h. Thereafter the combusted subsamples were weighed, and the Ash content was calculated.

3.3.6 Statistical Analysis

Statistical analysis on data was done using the statistical analysis software (SAS 2014). Where age effects were not a variable the statistics were done by using one-way analysis of variance (ANOVA) with Fisher least significant difference (LSD) *post hoc* test. Where age and treatment effects were variables the statistics were done using mixed model repeated measures of ANOVA with the Fisher LSD *post hoc* test. The 5% significance level was used for all statistical tests and treatment differences were declared at $P<0.05$.

The linear model used is described by the following equation:

$Yij = \mu + Ti + Hj + THij + eij$

Where Yij = variable studied during the period

μ = overall mean of the population

Ti = effect of the ith treatment

Hj = effect of the jth pen

THij = effect of the ijth interaction between treatment and house

eij = error associated with each Y

3.4 Results and Discussion

The results for the lipid evaluation test are shown below in Table 3.6. Free fatty acid content for soya oil was lower at 0.27% compared to the blended oil which was 10.63%. The unsaturated to saturated ratio (U/S) for soya oil was higher at 5.28% while for the blended oil it was 1.91%. The moisture, impurities and unsaponifiable matter (MIU) results for soya was below the recommended maximum level of 1.0% (Butolo, 2002) at 0.88%, and the blended oil was above the recommended level at 1.91%. This was in part due to the higher moisture levels of the blended oil which was 0.84% compared to soya oil which had a moisture content of 0.06%. The moisture was higher for the blended oil, although it was still below the recommended limit of 1.0% (Butolo,2002). The MIU value indicated possible dilution factors that did not contribute nutritionally to the oil, this could be seen in the unsaponifiable matter of the blended oil at 1.0%, which was at the limit (Butolo, 2002), while soya oil was 0.79%. Unsaponifiable matter is made up of natural components such as sterol and processed artifacts and cannot be digested. A higher saturated

fatty acid (SFA) value of 34.02% was found for the blended oil, compared to soya oil which was 15.9%. It was demonstrated by Wiseman *et al*. (1991), Leeson & Atteh (1995) and Smits *et al*. (2000) that fat utilization by the bird decreased with a higher SFA content. Van Kuiken & Behnke (1994) found that lipase activity was inhibited by long chain SFA, this could be the reason why the FCR was lower for the blended oil compared to soya oil (

Table **3.11**).

Table 3.6 Chemical analysis results (%) and AME calculation for blended oil and soya oil used in the trial

Parameters	**Blended Oil**	**Soya Oil**
Free fatty acid value (%)	10.63	0.27
Total saturated fatty acids (%)	34.02	15.90
Total unsaturated fatty acids (%)	64.99	84.03
Unsaturated/saturated ratio (%)	1.91	5.28
Moisture (%)	0.84	0.06
Insoluble Impurities (%)	0.08	0.03
Unsaponifiable matter (%)	1.00	0.79
MIU[1] (%)	1.91	0.88
AME 0-21 days broilers (MJ/kg)	30.78	36.69
AME > 21 days broilers (MJ/kg)	33.82	37.66

([1]) Moisture, insoluble impurities and unsaponifiable matter

Results from the lipid evaluation test in Table 3.6 showed AME values for young broilers, 0-21 days of age, was 36.69 MJ/kg for soya oil and 30.78 MJ/kg for the blended oil, a difference of 5.91 MJ/kg or 16.1% lower. The AME for older birds of > 21 days was 37.66 for soya oil and 33.82 MJ/kg for the blended oil, which was a difference of 3.84 MJ/kg or 10.2% lower. The differences between the two oils are due to their chemical composition as proposed by Murgeson (2013) and Wiseman (1999). Both oils had a higher AME value for the older birds, which confirms the effect of age on fat digestion and absorption (Leeson & Atteh, 1995; Melegy *et al*., 2010). The soya oil was a higher quality oil with lower free fatty acid (FFA), SFA and MIU, with higher unsaturated fatty acid (UFA) value and this could be seen in the difference of AME values between the two oils. For formulation purposes, the AME values used for soya oil was 36.69 MJ/kg and 30.78 MJ/kg for the blended oil. The soya oil AME value differed from the tabulated value according to CVB (2012), which is 34.95, while for the blended oil there was no tabulated values available for blended oil due to its unknown chemical composition.

The proximate analysis of the treatment diets are summarized in Table 3.7, Table 3.8 and for the starter, grower and finisher diets, respectively. The resulting crude protein levels were higher than the calculated crude protein levels, but this did not impact on the broiler production parameters. When comparing results from Table 3.10 for the starter phase (day 1 to day 14), there is no significant treatment differences for average live weight, weekly feed intake as well as cumulative feed intake with these lower crude protein levels. Crude fibre results were one percentage point lower than the formulation values, while there was no major difference between the analysed results for crude fat.

Table 3.7 Analysed proximate analysis of the trial starter diets on an as is basis, with inclusion of lysoforte extend dry (LEX) on CONS+ and CONBO+, respectively.

Parameters	Unit	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
Moisture	%	11.07	11.68	11.61	11.54	11.90	12.45
Dry matter	%	88.93	88.32	88.39	88.46	88.10	87.55
Ash	%	6.09	5.82	6.13	6.58	5.96	5.31
Crude Fat	%	4.92	3.96	4.03	5.75	4.22	3.86
Crude Fibre	%	3.79	3.54	3.27	3.55	3.32	3.06
Crude Protein	%	24.59	24.59	24.22	24.69	24.75	25.31

([1]) Control diet with soya oil, ([2]) Decreased AME of 0.25 MJ/kg with soya oil ([3]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX ([4]) Control diet with blended oil ([5]) Decreased AME of 0.25 MJ/kg with blended oil ([6]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

Table 3.8 Analysed proximate analysis of the trial grower diets on as is basis, with inclusion of lysoforte extend dry (LEX) on CONS+ and CONBO+, respectively.

Parameters	Unit	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
Moisture	%	11.26	10.75	11.95	11.25	11.33	12.15
Dry matter	%	88.74	89.25	88.05	88.75	88.67	87.85
Ash	%	5.10	4.99	4.61	4.77	5.16	4.53
Crude Fat	%	4.09	3.75	3.47	4.86	4.24	3.74
Crude Fibre	%	2.72	3.18	3.17	2.88	3.19	2.81
Crude Protein	%	22.38	21.78	21.88	22.34	22.03	21.63

([1]) Control diet with soya oil, ([2]) Decreased AME of 0.25 MJ/kg with soya oil ([3]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX ([4]) Control diet with blended oil ([5]) Decreased AME of 0.25 MJ/kg with blended oil ([6]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

The summarized results reported during the broiler growth performance trial is shown below (Table 3.10). No significant ($P<0.05$) treatment differences for average live weight, weekly feed intake and cumulative feed intake were observed up to day 14. At 21 days there was significant treatment differences observed for average live weight between the treatments where CONS,

CONS-, CONS+ and CONBO were significantly (P<0.05) higher than CONBO+. Significant differences (P<0.05) for weekly feed intake was observed in this study between soya oil and the blended oil diet CONS+ had significantly higher weekly feed intake than all other treatments. The average liveweight at day 28 was significantly lower for CONBO+ than all other treatments. Chicks that received CONS+ had significantly higher (P<0.05) feed intake when compared to CONS at day 28, while there was no significant difference between CONS+ and CONBO+ for weekly feed intake. No significant differences for cumulative feed intake was observed between the CONS+ and all diets with blended oil, however they were all significantly higher (P<0.05) than CONS.

Table 3.9 Analysed proximate analysis of the trial finisher diets on as is basis, with inclusion of lysoforte extend dry (LEX) on CONS+ and CONBO+, respectively.

Parameters	Unit	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
Moisture	%	11.97	11.94	11.61	12.71	12.37	11.66
Dry matter	%	88.03	88.06	88.39	87.29	87.63	88.34
Ash	%	4.32	4.67	4.64	4.55	4.41	4.59
Crude Fat	%	3.40	3.57	3.61	5.24	4.36	4.83
Crude Fibre	%	2.68	2.90	2.89	3.45	3.86	3.30
Crude Protein	%	20.79	21.25	21.22	20.73	20.75	20.84

([1]) Control diet with soya oil, ([2]) Decreased AME of 0.25 MJ/kg with soya oil ([3]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX ([4]) Control diet with blended oil ([5]) Decreased AME of 0.25 MJ/kg with blended oil ([6]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

At the end of the trial, no significant difference was observed between the soya oil treatments for average live weight, however they were all significantly heavier (P<0.05) than CONBO+. The average live weight for CONS+ was also significantly higher (P<0.05) than CONBO. While CONBO had a significantly heavier (P<0.05) average live weight than both CONBO- and CONBO+. Weekly feed intake showed no significant difference between the treatments for the last week of the trial. Cumulative feed intake for CONS+ was significantly higher (P<0.05) than the CONS as well as CONS-. All three blended oil treatments had a significantly lower (P<0.05) cumulative feed intake than CONS+.

Results from the trial in Table 3.11 below, showed no significant differences between the treatments for mortalities and ADG. Significant difference was observed for FCR, which was significantly lower (P<0.05) on CONBO+ than on both the CONS and CONBO. No significant differences were observed between the other treatment diets for FCR. There were no significant treatment differences observed in the trial for both PER and EPEF.

Table 3.10 Averages (± standard error) of weekly live weight (g), weekly feed intake (g), cumulative feed intake (g) and production ratios of broilers which received lysophospholipid with two different oil sources.

Parameters	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
Day 1						
Average Live Weight	45.37 ± 0.97	45.48 ± 1.02	45.42 ± 0.95	45.14 ± 0.81	44.90 ± 0.73	45.34 ± 0.96
Day 7						
Average Live Weight	200.5 ± 6.80	201.9 ± 5.89	203.7 ± 3.97	196.0 ± 9.48	200.5 ± 4.33	201.2 ± 7.68
Weekly Feed Intake	161.9 ± 5.68	165.4 ± 3.14	163.5 ± 3.76	161.0 ± 4.73	163.2 ± 5.03	165.5 ± 6.02
Cumulative Feed Intake	161.9 ± 5.68	165.4 ± 3.14	163.5 ± 3.76	161.0 ± 4.73	163.2 ± 5.03	165.5 ± 6.02
Day 14						
Average Live Weight	515.9 ± 16.18	512.3 ± 17.28	522.3 ± 10.43	506.5 ± 14.91	506.6 ± 12.51	506.5 ± 9.45
Weekly Feed Intake	400.8 ± 13.58	412.3 ± 13.00	413.9 ± 11.40	400.4 ± 8.13	396.9 ± 16.98	403.7 ± 8.55
Cumulative Feed Intake	562.6 ± 13.42	577.7 ± 13.39	577.4 ± 13.33	561.4 ± 9.91	560.1 ± 18.63	569.2 ± 10.43
Day 21						
Average Live Weight	1108.4^{a} ± 24.42	1106.0^{a} ± 24.24	1104.6^{a} ± 32.92	1102.9^{a} ± 27.93	1081.10^{ab} ± 31.61	1071.2^{b} ± 45.12
Weekly Feed Intake	818.7^{a} ± 29.87	818.2^{ab} ± 28.57	840.3^{b} ± 68.52	815.0^{a} ± 22.89	811.8^{a} ± 29.83	814.7^{a} ± 24.45
Cumulative Feed Intake	1381.3^{ab} ± 38.97	1395.9^{ab} ± 34.80	1417.8^{a} ± 77.42	1376.4^{ab}± 28.21	1371.9^{b} ± 34.25	1383.9^{ab}± 26.72
Day 28						
Average Live Weight	1804.9^{a} ± 84.31	1795.4^{a} ± 71.86	1813.4^{a} ± 56.17	1810.8^{a} ± 51.15	1801.1^{a} ± 44.20	1767.5^{b} ± 80.96
Weekly Feed Intake	1037.1^{a} ± 62.23	1056.4^{ab} ± 32.42	1074.4^{a} ± 44.81	1061.5^{ab}± 29.59	1074.6^{b} ± 32.72	1072.5^{ab}± 33.22
Cumulative Feed Intake	2418.4^{a} ± 79.04	2452.3^{ab}± 54.85	2492.2^{b} ± 57.85	2437.9^{b} ± 45.26	2446.5^{b} ± 51.86	2456.4^{b} ± 50.99
Day 35						
Average Live Weight	2515.9^{a} ± 66.53	2504.6^{a} ± 40.79	2508.2^{a} ± 35.06	2515.6^{a} ± 55.24	2496.6^{a} ± 40.14	2475.6^{b} ± 70.02
Weekly Feed Intake	1309.5 ± 101.02	1299.4 ± 68.09	1321.6 ± 62.81	1309.6 ± 71.34	1317.7 ± 85.66	1334.2 ± 56.56
Cumulative Feed Intake	3727.9^{a} ± 131.79	3751.7^{a} ± 95.53	3813.8^{b} ± 76.03	3747.5^{a} ± 92.28	3764.2^{a} ± 93.73	3790.6^{a} ± 81.38

(^{abcd}) Means with different superscripts within the same row differ significantly (P<0.05)

([1]) Control diet with soya oil, ([2]) Decreased AME of 0.25 MJ/kg with soya oil ([3]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX ([4]) Control diet with blended oil ([5]) Decreased AME of 0.25 MJ/kg with blended oil ([6]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

Table 3.11 Mortalities (%) and production ratios of broilers which received lysophospholipid with two different oil sources.

Parameters	CONS[5]	CONS-[6]	CONS+[7]	CONBO[8]	CONBO-[9]	CONBO+[10]
Cumulative Mortalities (%)	3.13 ± 3.20	2.84 ± 5.22	2.27 ± 3.32	2.56 ± 2.86	3.98 ± 4.35	2.56 ± 4.69
ADG (g/day)[1]	70.59 ± 1.90	70.26 ± 1.18	70.37 ± 1.01	70.58 ± 1.58	70.05 ± 1.15	69.44 ± 2.01
FCR[2]	1.53[a] ± 0.03	1.55[ab] ± 0.03	1.56[ab] ± 0.02	1.54[a] ± 0.03	1.57[ab] ± 0.03	1.58[b] ± 0.03
EPEF[3]	449.45 ±29.68	443.78 ± 35.09	444.05 ± 17.43	450.00 ± 19.09	430.94 ± 28.07	435.01 ± 25.26
PER[4]	3.49 ± 0.07	3.44 ± 0.07	3.43 ± 0.06	3.46 ± 0.08	3.44 ± 0.07	3.38 ± 0.06

([1]) ADG – average daily gain, ([2]) FCR – Feed conversion ratio, ([3]) EPEF – European production efficiency factor, ([4]) PER – Protein efficiency ratio.
([abcd]) Means with different superscripts within the same row differ significantly (P<0.05)
([5]) Control diet with soya oil, ([6]) Decreased AME of 0.25 MJ/kg with soya oil ([7]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX ([8]) Control diet with blended oil ([9]) Decreased AME of 0.25 MJ/kg with blended oil ([10]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

Nir *et al*. (1993) observed significant differences at 14 days for live weight when using emulsifiers with different fat sources, but these results were in contrast with the results obtained from this trial. No differences were observed in the first and second week for average live weight (Table 3.10), this can be due to the inadequate development of the digestive system of the young chick (Kroghdahl, 1985). The results for live weight at day 21 was in contrast with Zobac *et al*. (1998) who observed that body weight of birds fed diets containing lecithin increased. The treatment CONBO+ had a significantly lower live weight at day 21, while there were no significant treatment differences between the other treatments for live weight. This correlated with the results found by Azman & Siftici (2004) as well as Zavareie & Toghyani (2018), who both used lecithin supplements and indicated that the body weights of birds were not affected by lecithin supplementation. Emmert *et al*. (1996) observed that there was an improvement of body weight of young birds, the reason for their results indicated the role which phospholipids play in fat digestion through their emulsification properties as well as nutrient absorption by increasing micelle formation, resulting in improved growth performance in young birds (Schwarzer & Adams, 1996). At 35 days, CONBO+ average liveweight remained significantly lower, while CONS+ showed no significant differences. This was in contrast with San Tan *et al*. (2016) and Roy *et al*. (2010) who used exogenous emulsifiers and reported improved body weight gains. Melegy *et al*. (2010) also confirmed the result that the addition of lysolecithin significantly improved body weight gain. This contradictory result on growth performance could be due to the degree of saturation of the blended oil when compared to soya oil, which has lower saturated FA content.

Zampiga *et al*. (2016) confirmed the results obtained on the soya oil treatments reporting that there was no significant difference on final body weight with the addition of an emulsifier showing that the effect of emulsifiers is less significant on unsaturated fat sources (Huyghebaert, 2003; Jansen 2015). The Ross 308 performance objectives at 35 days stated that the live weight of 2283 g should be achieved; in this trial (Table 3.10) all treatments achieved this live weight goal (Aviagen group, 2014[a]).

The results from the trial (Table 3.10) showed a higher cumulative feed intake on the diets containing additional LEX on both oils (CONS+ and CONBO+) when compared to other treatments with the same oil. These results coincide with the findings from Siyal *et al*. (2017), Roy *et al*. (2010) and Zosangpuii *et al*. (2011), who all used exogenous emulsifiers and observed a higher feed intake on these treatments. Zaeferian *et al*. (2015) who used 3.5 kg/ton lysophospholipid, also observed a significant increase in feed consumption. The positive effect on feed intake when an emulsifier is added could be because of an improved palatability, which led to a higher feed and energy intake (Cho *et al*., 2012). These results however contradict the results observed by Guerreiro *et al*. (2011), Aguilar *et al*. (2013) and Zhang *et al*. (2011) who used casein, a nonionic and lysophosphatidyl-choline emulsifier, respectively and observed no significant effect on feed intake. These results may be attributed to the improved nutrient digestibility when of a fat emulsifier is included, which may result in the fulfilment of the caloric requirements of the birds and hence the feed intake doesn't increase (Mathlouthi *et al*., 2002).

Trial results for ADG showed no significant differences between treatments and was comparable with reports from Zampigy *et al*. (2016) who also observed no significant difference on ADG when using an emulsifier at 1 kg/ton. This was however in contrast with results from Melegy *et al*. (2010) who used lysolecithin at an inclusion of 0.25 and 0.5 kg/ton and they had significantly higher ADG results when an emulsifier was added. Although these ADG results did not differ between treatments, they were all highre than the suggested ADG of 65 g/day for acceptable production as proposed by Butcher & Nilipour (2015). The effect of the LEX used in this trial showed that even when the dietary energy was reduced on CONS+ and CONBO+, the enhanced fat digestion and facilitated emulsification resulted in an improved fat absorption. This resulted in increased energy utilization and assisted in the absorption of other soluble nutrients and vitamins, recovering the reduced dietary energy (Melegy *et al*, 2010).

From the trial results (Table 3.11), it was expected that the FCR will be higher for the treatment diets where LEX was used, as observed by Melegy *et al*. (2010), Siyal*et al*. (2017), Roy *et al*. (2010), Zampiga *et al*. (2016) and Zosangpuii *et al*. (2011), when exogenous emulsifiers were

used. However, in this trial there was no significant effect on soya oil diets. In fact for CONBO+, the opposite was achieved as the FCR was significantly lower than both the control diets for the two oils. The reason for this higher FCR on the blended oil was as demonstrated by Van Kuiken & Behnke (1994), due to the inhibition of the lipase activity in the presence of long chain SFA present in the blended oil. The performance objectives for Ross 308 is an FCR of 1.54 at day 35, in this trial only the two control diets managed to achieve this target, although Butcher & Nilipour (2015) stated that an FCR of 1.75 was acceptable for a profitable operation and all treatments had managed to achieve this FCR result.

Although there were no significant differences observed between the treatments for PER, all the treatments had PER values above the optimum value of 3:1 (Wilding *et al*., 1968), which showed all treatments were able to utilize their dietary proteins efficiently. Butcher & Nilipour (2015) suggested production efficiency values that were acceptable to obtain normal production under optimal management, with adequate nutrition and suggested an EPEF of 360 needs to be achieved. From the results obtained in this study for EPEF, all the treatments were above these standards for acceptable normal production.

3.5 Conclusion

The results reported in this study where LEX was added showed no significant differences between soya oil treatments for live weight even with the decreased energy values, except for CONBO+, where a significant lighter live weight was observed. Cumulative feed intake was observed to be significantly higher for both CONS+ and CONBO+, while no significant differences were observed for ADG, PER and EPEF across all treatments. The FCR results showed no significant differences between the three treatments for soya oil, however, CONBO+ was significantly lower. Further research and trials are warranted on the use of emulsifiers, even though there were not many significant differences observed, the production parameters were higher than the standard for optimal broiler production even with the reduced energy in four of the treatment diets. The results proved the potential to decrease the energy content of the feed when adding LEX in the diet without adverse effects on production parameters of broilers.

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Chapter 4

The effect of two different oil sources and addition of a lysophospholipid on the organ and carcass characteristics of broiler chickens

4.1 Abstract

A 35-day experiment was conducted to investigate the effect two oils and the inclusion of a lysophospholipid in broiler diets had on organ weight, gizzard erosion score, breast meat pH and carcass characteristics. For each type of oil, three diets were formulated, the first with standard AME (CONS and CONBO) and two containing 0.25 MJ/kg less energy (CONS- and CONBO-). One of the reduced energy diets per type of oil, included Lysoforte Extend Dry (LEX), the lysophospholipid, at an inclusion level of 500 g/ton (CONS+ and CONBO+). The two oils used was refined soya oil and a lower quality unsaturated blend of animal fats and vegetable oils. Two thousand one hundred and twelve chicks were randomly allocated to one of six treatments with sixteen replicates per treatment. At 35 days of age, one bird per pen was slaughtered for data collection. No significant differences were observed for the blended oil on the weights of the bursa of Fabricus, gizzard, heart, liver or spleen, however, for soya oil treatments the weight of the liver was significantly higher when LEX was added, while the spleen weight was significantly lower than the control. Gizzard score for the treatment with CONS+ was significantly higher than the CONS and CONBO. Significant differences were observed for breast weight and relative breast weight, where the CONS+ was significantly lower than CONS for both oils. The addition of LEX to the diet did not influence the dressing percentage, carcass weight, carcass portion weights (wings, thighs and drumsticks), relative carcass portion weights, relative right breast portions (skin and fat, muscle and bone), breast pH warm and breast pH chilled after 24 hours post mortem. Therefore, it can be concluded that adding LEX to the diet of broilers with a decreased AME value, had no adverse effects on the organ or carcass characteristics of the broilers.

Keywords: poultry, gizzard, heart, liver, spleen, carcass portions, muscle pH, Lysoforte Extend dry

4.2 Introduction

The oils and fats of natural resources are incorporated in poultry feed to enhance the energy contents of the diets (Siyal et al., 2017). Lipids provide the main source of energy to animals and

have the highest caloric value among all nutrients (Zhao & Kim 2017). It has been well documented that the body composition of broilers is influenced by energy intake (Boekholt et al., 1994; Wiseman and Lewis, 1998). Lipids form an important component and performs vital functions within the animal's body. The use of exogenous emulsifiers can positively impact the performance of the birds given the amount of lipids added to broiler diets (Zampiga *et al*., 2016). The addition of synthetic emulsifier to broiler diets is a recent practice as compared to other dietary supplements. The mode of action of emulsifiers is to increase the active surface of fats, allowing the action of lipase, which hydrolyse triglyceride molecules into fatty acids and monoglycerides and favours the formation of micelles consisting of lipolysis products. This is an essential step for lipid absorption, as it creates a diffusion gradient that increases absorption (Guerreiro Neto *et al*., 2011). The supplementation of soy lecithin alone or in combination with lipase enzyme in broilers diets (100 U/kg feed) exerted beneficial effects on performance, carcass quality, oxidative stability, and thereby increasing shelf life of meat during refrigerated storage and thus also profitability (Nagargoje *et al*., 2016).

The pH of meat has a direct influence on meat quality attributes such as tenderness, water-holding capacity, colour, juiciness and shelf life. An increased water binding capacity of broiler meat is found where the meat had a higher pH. The pH of broiler meat is a function of the amount of glycogen in the muscle before slaughter and the rate of glycogen conversion into lactic acid post slaughter. The use of colour is an easy way to determine the pH of meat. It was found that darker coloured meat had higher pH values than lighter coloured meat (Anadon 2002). The shelf life of this darker meat was reduced, which can be due to an increased number of psycotropic bacteria that colonize the darker meat (Allen et al., 1998). Poultry meat with low pH has been associated with low water-holding capacity, which results in increased cook-loss, drip loss, shelf-life and decreased tenderness (Barbut 1993; Qiao et al., 2001).

The immune cell hubs are formed by the spleen, thymus and bursa; production and orientation of these immune cells occur more efficiently in these organs in healthy animals as opposed to immune compromised animals (Sikandar *et al*., 2017). In disease free, healthy birds, the increased weight of the immune organs correlates with an increased production of immune cells and ultimately result in improved immunity (Teo & Tan, 2007). A healthy spleen has a stimulatory effect on the production of B cells, which have the potential to improve the immune status of the bird through immunoglobulin synthesis. In avian species, the main organ of the immune system is the bursa of Fabricius (Sikandar *et al*., 2017). The liver plays an important role in digestion and metabolism, regulating the production, storage, and release of lipids, carbohydrates and proteins (Denbow, 2000). The liver also contributes to the immunity of the bird through synthesizing

accessory proteins (Sikandar *et al*., 2017). The liver plays the main role in lipogenesis, providing lipids destined to be used by all tissues including by the liver itself. Fats that are metabolised in the liver are derived from three main sources: dietary fat, depot fat and fat from *de novo* fatty acid synthesis, which is derived from carbohydrates in the feed (Hermier, 1997).

The effect of lysophospholipids on organ characteristics of broilers have been well documented, however many of these results are contradicting. The trials of Andreotti *et al*. (2004), Ferreira *et al*. (2005), Lara *et al*. (2006), Roy *et al*. (2010), Guerreiro Neto *et al*. (2011), Cho *et al*. (2012) and Abbas *et al*. (2016) showed no significant differences on organ weights when emulsifiers were added to broiler diets. This is in contrast to Praharaj *et al*. (1997); their results showed a significant difference between the weights of the liver, heart, spleen and gizzard when emulsifiers were added to broiler diets. Similarly, Huang *et al*. (2007), Nagargoje *et al*. (2016) and Siyal *et al*. (2017) observed the liver weights to be significantly heavier when soy lecithin was added to the diet. The reason for the increased liver weight may be due to the increased metabolic activity related to lipid utilization (Al-Marzooqi & Leeson, 2000).

Genetic selection among all breeds of broilers has resulted in an improvement of carcass characteristics. The most important has been the yield of the more expensive cuts. These cuts include the breast muscle and the legs (Fernandes *et al.*, 2013). It is important to look at the effect LEX has on all portion sizes. This is because the wholesale prices per kilogram differs (SAPA, 2013) between portions and this may affect profits if chickens are sold as commercial cuts. Various trials using fat emulsifiers on carcass portions have showed that the inclusion of fat emulsifiers had no significant effect on the carcass portion yields (Andreotti *et al*., 2004; Ferreira *et al*., 2005; Lara *et al*., 2006; Melegy *et al*., 2010; Guerreiro Neto *et al*., 2011; Aquilar *et al*., 2013; Zampiga *et al*., 2016.

However, broilers fed diets containing various fat emulsifiers, was shown by Melegey *et al*. (2010) to result in an increased dressing percentage. On the other hand, Guerreiro Neto *et al*. (2011), Cho *et al*. (2012), Aquilar *et al*. (2013) and Zampiga *et al*. (2016) showed there were no significant differences on the dressing percentage when fat emulsifiers were added to the diet. The inconsistent results may be attributed to the variation of the diets, the lysophospholipid inclusion and on level of fat content of the diet.

The aim of this study was to investigate the effect of supplementing two different oils with a lysophospholipid and its effect on organ and carcass characteristics of broiler chicks.

4.3 Materials and Methods

4.3.1 Experimental layout, handling and management

The detailed description of the experimental procedure, handling of the broiler chicks and their management throughout the trial is outlined in Chapter 3 under sections 3.3.2 Experimental diets and 3.3.3 Animals and housing system respectively. Briefly, the trial was conducted on 2112 Ross 308 chicks and only males were used. The birds were randomly allocated to six treatments, with sixteen replicates per treatment using two oils, namely soya oil and blended oil. Each oil investigated had three treatments, where two treatments had a reduced dietary energy value of 0.25 MJ/kg and one of these decreased energy treatments had 500 g/ton LEX included. The broiler chickens were raised until slaughter at day 35 of age.

4.3.2 Slaughtering procedure

At the age of 35 days, all birds from each of the 96 pens were weighed to attain the average weight of each pen. One bird was randomly selected from each pen, the bird's weight was recorded and thereafter each bird was marked with a specific coloured zip tie. Each treatment had the same colour zip tie attached to the leg of the randomly selected birds. The slaughtering process took place at the Sovereign abattoir in Uitenhage where all their commercial broilers are slaughtered and processed. The slaughtering procedure was done as per the acceptable slaughtering standard method (Department of Agriculture, Forestry & Fisheries (DAFF), 2006). At slaughter the birds were rendered unconscious through electrical stunning (50-70 volts; 3-5 s), exsanguinated and bled out for about two minutes. Following this the birds were soaked in a 53.5 °C water bath for approximately two and a half minutes, after which birds were defeathered and then eviscerated (this included the removal of all the internal organs, feet and neck).

4.3.3 Organ sampling

Organs removed for this trial included the bursa of Fabricius, gizzard, heart, liver and the spleen. The gizzards were cut open and rinsed with clean water and scored for gizzard erosion, using an ordinal scale according to Johnson & Pinedo (1971), as shown in Table 4.1 . The organs were vacuum sealed, frozen and sent to the Stellenbosch University meat laboratory for further analysis. All organs were weighed on a PC 400 Mettler laboratory scale (Mettler-Toledo, Switzerland).

Table 4.1 Gizzard erosion scoring and description (Johnson & Pinedo, 1971)

Score	Description
0	No erosion
1	Light erosion (minimal roughness of the epithelia)
2	Modest erosion (roughness and minimal gaps of the epithelia)
3	Severe erosion (roughness, gaps and ulcers on the wall showing slight haemorrhaging)
4	Extreme erosion (roughness, gaps and haemorrhagic ulcers on stomach wall and visible separation of epithelia from the stomach wall

4.3.4 Carcass characteristics

After the carcass had been eviscerated, each carcass was weighed to determine the carcass weight. Dressing percentage was calculated as the percentage difference between the live weight of the chicken and the weight of the hot carcass. The carcasses were then portioned into commercial cuts (breast, drumstick, thigh and wing) using a commercial meat slicer. The cutting procedure was as follows: firstly, the whole carcass was halved into two. Then, the leg was removed by cutting above the thigh towards the acetabulum just behind the pubic bone. The leg was further cut perpendicular to the joints where the tibia, fibula and femur bones are attached together to obtain the drumstick and thigh portions. Then the wing was removed by cutting through the joint between the scapula and coracoid and the breast portion was separated from the wing. These portions were then weighed in pairs and their weights recorded. The right breast portion was weighed and then skinned, deboned and the subcutaneous fat cover was removed. Each of these portions were individually weighed to determine the percentage of each of the total right breast weight.

4.3.5 pH measurement

Within 15 min after the slaughtering procedure started, the pH measurement of the right breast of each bird was taken. A handheld, portable Crison pH 25 meter (Alella, Barcelona) was used to take these measurements. Before the slaughter process started the Crison 25 was calibrated with standard pH buffers (pH 4.0 and pH 7.0) as provided by the manufacturer. The probe was inserted into the breast muscle and the reading was allowed to settle before it was recorded. Between each measurement the probe was rinsed with distilled water and rested in a 3M KCl electrolytic solution. The right breast was immediately chilled for 24 hours at 4 °C on site, after 24 hours the pH reading was recorded again.

4.3.6 Statistical analysis

Statistical analysis was done using the general linear models (GLM) procedure of SAS (2014). The analysis of variance (ANOVA) assumptions for normality and homoscedasticity were investigated before further analyses were done. The tests were considered significant at $P<0.05$. Treatment effects of all parameters except for gizzard erosion score were analysed using one-way ANOVA with Bonferroni's *post hoc* (least square means) test. In cases where the homoscedasticity assumption for the data was not satisfied, a Welch's ANOVA for unequal variances was used. The significance level of 5% of all tests was used and significant treatment differences were declared at $P<0.05$.

The linear model used is described by the following equation:

$Yij = \mu + Ti + Hj + THij + eij$

Where Yij = variable studied during the period

μ = overall mean of the population

Ti = effect of the ith treatment

Hj = effect of the jth pen

THij = effect of the ijth interaction between treatment and house

eij = error associated with each Y

4.4 Results and Discussion

Table 4.2 summarizes the results obtained from the different treatments on the organ characteristics of the broilers. There were no significant differences observed between CONS+ and CONBO+ on organ weight as well as relative organ weight for the gizzard, bursa of Fabricius and the heart. The gizzard erosion score also showed no significant differences between the two oils when LEX was added. The gizzard weight for CONS was lower ($P<0.05$) than all other treatment diets. There was no significant difference between the other treatments for gizzard weight. The bursa of Fabricius weight for CONS- was lower ($P<0.05$) when compared to the CONBO. There were no other treatments differences observed for bursa of Fabricius weight.

Table 4.2 Mean (± standard error) of organ weight, organ weight relative to carcass weight and gizzard erosion scores as influenced by the two different oils with the inclusion of a lysophospholipid in broilers

Parameters	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
Organ weight (g)						
Gizzard	25.72[b] ± 3.42	30.50[a] ± 3.56	30.19[a] ± 4.41	30.11[a] ± 5.36	30.91[a] ± 4.32	32.11[a] ± 3.42
Bursa	1.21[ab] ± 0.24	1.05[a] ± 0.27	1.25[ab] ± 0.33	1.32[b] ± 0.33	1.19[ab] ± 0.22	1.12[ab] ± 0.27
Liver	52.47[a] ± 5.39	51.36[a] ± 7.44	58.26[b] ± 15.84	51.81[a] ± 4.49	55.17[ab] ± 4.83	51.73[a] ± 4.97
Heart	11.49[ab] ± 1.58	11.53[ab]± 1.46	10.47[a] ± 1.45	11.76[b] ± 1.75	11.52[ab] ± 1.73	10.97[ab]± 1.11
Spleen	2.89[a] ± 0.60	2.71[a] ± 0.59	3.33[b] ± 0.81	2.73[a] ± 0.57	2.89[a] ± 0.79	2.51[a] ± 0.47
Bursa:Spleen	0.44[ab] ± 0.15	0.39[b] ± 0.12	0.39[b] ± 0.11	0.52[a] ± 0.12	0.42[b] ± 0.10	0.45[ab] ± 0.12
Organ weight relative to carcass weight (%)						
Gizzard	1.49[b] ± 0.06	1.74[a] ± 0.06	1.77[a] ± 0.06	1.71[a] ± 0.06	1.78[a] ± 0.06	1.86[a] ± 0.06
Bursa	0.07 ± 0.005	0.059 ± 0.005	0.075 ± 0.004	0.074 ± 0.004	0.069 ± 0.004	0.066 ± 0.005
Liver	3.06[b] ± 0.12	2.93[b] ± 0.12	3.41[a] ± 0.12	2.93[b] ± 0.12	3.17[ab] ± 0.12	2.99[b] ± 0.12
Heart	0.67 ± 0.02	0.66 ± 0.02	0.62 ± 0.02	0.67 ± 0.02	0.66 ± 0.02	0.63 ± 0.02
Spleen	0.17[b] ± 0.01	0.15[b] ± 0.01	0.20[a] ± 0.01	0.15[b] ± 0.01	0.17[b] ± 0.01	0.15[b] ± 0.01
Gizzard score	1.38[a] ± 0.50	1.69[ab] ± 0.87	2.25[b] ± 1.34	1.5[a] ± 0.63	1.56[a] ± 0.63	1.69[ab] ± 1.01

([abcd]) Means with different superscripts within the same row differ significantly (P<0.05)
([1]) Control diet with soya oil, ([2]) Decreased AME of 0.25 MJ/kg with soya oil ([3]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX ([4]) Control diet with blended oil ([5]) Decreased AME of 0.25 MJ/kg with blended oil ([6]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

The results on liver weight, showed no significant difference between CONS, CONS-, CONBO, CONBO- and CONBO+. However, CONS+ had a significantly higher liver weight than all other treatments. The weight of the heart in CONS+ was significantly lower than CONBO. No significant treatment differences were observed between the other treatments. Spleen weight of CONS+ was significantly higher than all the other treatment diets. The ratio of bursa to spleen was significantly lower for CON- and CONS+ and CONBO- compared to CONBO. Relative organ to carcass weight showed no significant treatment differences for the bursa of Fabricius and the heart. For the gizzard as a percentage of carcass weight, CONS was significantly lower than all other treatments. The relative liver weight results were significantly higher for CONS and CONS- compared to CONS+, while CONS+ was also significantly higher than CONBO+. On the spleen to carcass weight CONS+ was significantly higher than CONBO+, while there was no significant difference

between the blended oil treatments. Gizzard erosion score for CONS was significantly lower than CONS+, while CONBO and CONBO- was significantly lower than CONS+.

Table 4.3 summarizes the influence of treatment with lysophospholipids with two different oils on the live weight, carcass weight and dressing percentage of broilers. The CONBO treatment had a significantly higher live weight compared to CONS and CONS+. No other treatment differences were observed. The CONBO diet also had a significantly heavier carcass compared to the CONS and CONS+, while CONS- results were also significantly higher than CONS+. The heavier carcass weight can be explained from the heavier liveweight of the randomly selected birds of the different treatments. No significant treatment differences were observed for dressing percentage between the treatment diets.

Table 4.4 depicts the portion yields as well as portion yields as a percentage of the carcass weight, no significant treatment differences were observed for the weight of the wings, similarly the same was observed for the wings as a percentage of carcass weight. The thigh weights of CONBO- was significantly lower than CONBO. The relative portion weight to carcass weight on the thighs showed CONBO- was significantly lower than CONS+ and CONBO. The drumstick weights as well as relative to the carcass weight for CONBO- was significantly higher than CONBO+. The other treatment results for the thighs and drumsticks showed no significant differences. The breast weight of CONS+ was significantly lower than CONS, CONS-, CONBO and CONBO-. The CONS- treatment had a significantly higher breast weight compared to CONBO+. The relative breast muscle weight to carcass weight was significantly lower for CONS+ compared to CONS and CONS-.

Results for the skin and fat, bone and muscle portion weight as well as the breast portions as a percentage of the right breast is summarized in Table 4.5. No significant treatments differences were observed for the skin, fat and muscle portions of the breast and for the muscle portions percentages. The CONS- had the largest portion of bone in the breast and was significantly higher than all the other treatments.

Table 4.3 The means (± standard error) of live weight at slaughter, carcass weight and dressing percentage of broilers as influenced by the two different oils with the inclusion of a lysophospholipid

Parameters	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
Live slaughter weight (g)	2504.06[a] ± 64.62	2526.75[ab]± 45.69	2492.63[a] ± 69.81	2553.44[b] ± 57.12	2521.31[ab]± 62.31	2509.56[ab]± 75.14
Carcass weight (g)	1720.38[a] ± 69.89	1753.5[b] ± 64.06	1706.38[a] ± 53.39	1768.88[b] ± 46.15	1740.25[ab]± 56.63	1730.81[ab]± 66.10
Dressing percentage (%)	68.68 ± 1.39	69.4 ± 2.29	68.46 ± 1.25	69.29 ± 1.85	69.02 ± 1.37	68.97 ± 1.42

([abcd]) Means with different superscripts within the same row differ significantly (P<0.05)

Table 4.4 The means (± standard error) of broiler carcass portion yield and the portion yield as a percentage of carcass weight as influenced by the two different oils with the inclusion of a lysophospholipid

Parameters	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
Wings (g)	169.69 ± 15.82	176.06 ± 9.79	171.38 ± 10.16	175.44 ± 13.36	166.88 ± 31.59	169.88 ± 24.69
Thighs (g)	450.94[ab] ± 39.25	460.13[ab] ± 35.47	459.19[ab] ± 24.9	476.44[a] ± 36.81	431.06[b] ± 70.6	460.19[ab] ± 28.71
Drumsticks (g)	233.56[ab] ± 14.64	232.44[ab] ± 19.38	235.31[ab] ± 6.11	239.88[ab] ± 15.48	250.25[b] ± 50.18	229.25[a] ± 17.48
Breast (g)	644.88[b] ± 45.69	661.13[b] ± 42.31	610.69[a] ± 37.84	644.56[b] ± 46.15	643.25[b] ± 37.44	631.13[b] ± 36.51
Wings (%)	9.88 ± 0.95	10.05 ± 0.53	10.05 ± 0.66	9.92 ± 0.65	9.59 ± 1.76	9.8 ± 1.25
Thighs (%)	26.20[ab]± 1.69	26.24[ab]± 1.77	26.92[a] ± 1.39	26.95[a] ± 2.08	24.75[b] ± 3.74	26.6[a] ± 1.56
Drumsticks (%)	13.59[ab]± 0.88	13.26[b] ± 1.04	13.79[ab]± 0.76	13.56[ab]± 0.78	14.39[a] ± 2.83	13.24[b] ± 0.72
Breast (%)	37.48[a] ± 2.05	37.69[a] ± 1.65	35.78[b] ± 1.61	36.44[ab]± 2.31	36.97[ab]± 1.89	36.47[ab]± 1.77

([abcd]) Means with different superscripts within the same row differ significantly (P<0.05)

([1]) Control diet with soya oil, ([2]) Decreased AME of 0.25 MJ/kg with soya oil ([3]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX ([4]) Control diet with blended oil ([5]) Decreased AME of 0.25 MJ/kg with blended oil ([6]) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

Table 4.5 The means (± standard error) for skin, muscle and bone percentage of the right portion of the breast as influenced by the two different oils with the inclusion of a lysophospholipid

Parameters	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
Skin plus fat (%)	3.75 ± 1.84	3.94 ± 1.36	3.64 ± 1.23	3.45 ± 0.94	3.87 ± 0.88	3.58 ± 0.88
Muscle (%)	38.18 ± 1.70	37.31 ± 2.23	37.32 ± 2.34	36.99 ± 2.16	37.87 ± 1.97	37.00 ± 1.96
Bone (%)	7.82^{a} ± 2.05	9.93^{b} ± 4.75	8.44^{ab}± 2.16	8.17^{ab}± 1.92	8.80^{ab}± 2.17	7.78^{a} ± 2.11

(^{abcd}) Means with different superscripts within the same row differ significantly (P<0.05)

(^{1}) Control diet with soya oil, (^{2}) Decreased AME of 0.25 MJ/kg with soya oil (^{3}) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX (^{4}) Control diet with blended oil (^{5}) Decreased AME of 0.25 MJ/kg with blended oil (^{6}) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

The pH results obtained on this trial are summarized in Table 4.6 below. No significant treatment differences were observed for the initial and ultimate pH of the breast muscle.

Table 4.6 The means (± standard error) for physical measurements of initial and ultimate pH of the breast muscle as influenced by the two different oils with the inclusion of a lysophospholipid

Parameters	CONS[1]	CONS-[2]	CONS+[3]	CONBO[4]	CONBO-[5]	CONBO+[6]
pH_i	5.99 ± 0.20	5.95 ± 0.19	6.02 ± 0.26	6.01 ± 0.18	5.98 ± 0.17	6.03 ± 0.19
pH_μ	5.90 ± 0.12	5.90 ± 0.15	5.85 ± 0.10	5.91 ± 0.14	5.85 ± 0.12	5.88 ± 0.15

($_i$) initial pH

(μ) ultimate PH

(^{1}) Control diet with soya oil, (^{2}) Decreased AME of 0.25 MJ/kg with soya oil (^{3}) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX (^{4}) Control diet with blended oil (^{5}) Decreased AME of 0.25 MJ/kg with blended oil (^{6}) Decreased AME of 0.25 MJ/kg with 500 g/ton LEX

The addition of LEX did not result in significant differences on the gizzard or bursa of Fabricius weight on both oils used, which correlates with the results observed by Cho *et al*. 2012, Abbas *et al*. (2016), Andreotti *et al*. (2004), Roy *et al*. (2010), Luc *et al*. (2013), Ferreira *et al*. (2005), Lara *et al*. (2006) and Guerreeiro Neto *et al*. (2011), who all observed, when using emulsifier supplementation on broilers, there was no significant difference on organ weights. The reason for this according to Nobakht (2011), was due to improved utilization of fat as an energy source when emulsifiers are added to the diet, making up for the decrease in dietary energy. The only lymphoid organ in avian species to act as both a primary and secondary lymphoid organ is the bursa of Fabricius. B-cells are produced in the bursa of Fabricius, which are responsive towards antigens and ultimately provide immune protection for the birds. Due to this vital role which the bursa of Fabricius plays in the immune health of the birds, it's essential to evaluate the effect of supplementing LEX on the bursa of Fabricius and in this trial, there was no significant impact on the weight of the bursa of Fabricius. An increase in the lymphoid organ weight can be considered as an indication of an improvement of the immune system (Nourmohammadi *et al.,* 2011), but it is important to keep in mind that an excessive or inappropriate immune response will unnecessarily depress performance of the birds (Collett *et al.,* 2005).

The results from Praharaj *et al*. (1997), showed a significant difference of the liver, heart, spleen and gizzard when an emulsifier was used in the diet of broiler chicks. This corresponds to the results obtained from this study where it was found that there were significant differences when LEX was added on the heart, liver and spleen weights on the soya oil treatments, however there was no significant difference on the heart, liver and spleen weights when LEX was added in the blended oil treatments. The liver was significantly larger in CONS+ than in the CONS and CONS- treatments. These results correlate with findings by Siyal *et al*. (2017), Huang *et al*. (2007) and Nagargoje *et al*. (2016) who all observed the liver to have a higher weight when adding a soy lecithin into the diet. Similar results were reported by Al-Marzooqi & Leeson (2000); they found the percentage weight of the liver was increased with lipase supplementation in broilers fed diets containing 4% animal-vegetable blended fat on day 21. Lipid metabolism occurs predominantly in the liver and up to 95% of *de novo* fatty acid synthesis occurs here (Theil & Lauridsen, 2007). Therefore, the increased weight in CONS+ can indicate increased metabolic activity related to a more improved lipid utilization (Al-Marzooqi & Leeson, 2000).

Gizzard erosion score analyses the occurrence of possible lesions or a change within the lining of the gizzard as effected by the different treatment diets with the two oils used. Damage to the gizzard is mostly seen as a rough inner lining of the gizzard or in more severe cases, erosions and ulcerations of the inner muscle can occur (Wessels & Post, 1989). The gizzard

score CONS+ was significantly higher than both CONS and CONBO. According to Johnson & Pinedo (1971), an acceptable gizzard erosion score is between zero and two, while three and above is not acceptable. Even though the CONS+ was significantly higher it was still acceptable and shows that the addition of LEX with both oils had no adverse effect on the gizzard lining.

Carcass dressing percentage is influenced by muscle growth and/or visceral growth. Dressing percentage decreases when abdominal fat, which is considered as a waste in broiler production, or visceral organ weight, increases (Salma *et al*., 2007). The results from this trial showed no significant treatment differences for dressing percentage between the different treatments. This is similar to the findings of Zampiga *et al*. (2016), Guerreiro Neto *et al*. (2011) and Aquilar *et al*. (2013), who used various fat emulsifiers in the diets and reported that the addition of the emulsifier did not have any effect on the dressing percentage of broilers. The findings of Cho *et al*. (2012) and Zavareie & Toghyani (2018), who used sodium steroyl-2-lactylate and a phospholipid respectively, also observed no difference on carcass dressing percentage. In contrast, Melegy *et al*. (2010) showed a significant increase of dressing percentage when birds were supplemented with Lysoforte Booster in comparison to the control group.

There were no significant treatment differences observed in this trial for the wings, thighs and drumstick portions in agreement with Melegy *et al*. (2010), who found no significant difference between breast and thigh weight when Lysoforte Booster emulsifier additive was used. Further, Andreotti *et al*. (2004), Ferreira *et al*. (2005), Lara *et al*. (2006), Guerreiro Neto *et al*. (2011), Aquilar *et al*. (2013) and Zampiga *et al*. (2016) reported no differences in carcass portions when different fat sources or emulsifier were used in broiler diets. However, drumsticks weight of CONS- was significantly higher than the drumsticks of CONBO+ (Table 4.4), although for the weights relative to carcass weights there were no significant differences. From the results of this trial it was found that both CONS+ and CONBO+, had significantly lower breast weights, compared to the other diets. On the relative portion weight however CONS+ was significantly lower than both the soya oil treatments. The contrasting carcass results can be affected by the emulsifier source as well as dietary composition (Boontiam *et al*., 2016). Fat source is important - findings from Lara *et al*. (2006), showed that vegetable fats don't influence carcass characteristics of broilers.

There were no significant treatment differences for the warm and 24-hour chilled breast pH values between treatments. It was reported by Van Laack *et al*. (2000) that the normal pH of breast meat should be at a pH of 5.96, all the 24-hour chilled pH averages were below this normal value. Therefore, the addition of LEX to the diet did not influence the pH values of the

breast muscle. In agreement, Upadhaya *et al*. (2017), observed that a reduced energy diet (0.42 MJ/kg) had no effect on the pH value in broilers fed diets containing 1.25% to 3.61% tallow. Selection of the modern broiler for higher body weight and increased lean muscle has induced histological and biochemical modifications of the muscle tissue which can ultimately lead to poor quality meat (Barbut et al., 2008; Petracci & Cavani, 2011). After slaughter, during the conversion of muscle to meat, anaerobic glycolysis results in a pH decline within the muscle. The rate and extent of this pH decline are important determinants of meat quality. Initial pH is determined within one hour after slaughter while ultimate pH is taken 24 hours post mortem. If the initial pH is already below 5.8, the meat may be pale, soft and exudative (PSE) as the pH dropped too low too quickly and will have a lower water-holding capacity (Van Laack et al., 2000). On the other hand, if the ultimate pH is above 6.3 the meat may be classified as dark firm and dry (DFD) as the pH did not drop to normal levels (Van Laack, 2000), this phenomenon is associated with darker colour, reduced drip loss and increased firmness (Fletcher, 1999; Richardson & Mead, 1999). This selection for increased lean muscle yield and decreased fat deposition results in effects on muscle metabolism through reduced glycogen storage and thus decreased post mortem acidification (Berri et al., 2005). It was reported by Nagargoje et al. (2016), that the supplementation of soy lecithin alone or in combination with lipase enzyme in broilers diets (100 U/kg feed) exerted beneficial effects on performance, carcass quality, oxidative stability, and ultimately increases shelf life of meat during refrigerated storage.

4.5 Conclusion

The results in this study with inclusion of LEX while decreasing the AME value on two different oil sources, had no effect, positively or negatively on the gizzard, bursa or heart weights. However, on the liver and spleen weights as well as gizzard score, the LEX added with soya oil resulted in significantly higher weights and gizzard erosion score, while the LEX added with the blended oil showed no significant difference between treatments. It can therefore be concluded that the addition of LEX to both soya oil and the blended oil had no adverse effect on organ weights of broilers. The cheaper lower quality blended oil can be used instead of soya oil with no effect on organ weights. On carcass characteristics, the addition of LEX showed no difference between treatments, except the breast portion of both oils with LEX that were observed to have lower weights than the other treatment diets. The results thus showed that when the AME of the diet was decreased and LEX was added at a rate of 500 g/ton feed, using either soya oil or the blended oil, there was no effect on the carcass traits and shows its viability in broiler production.

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Chapter 5

General conclusion

Lipid quality differs between vegetable, animal and blended oils. Oils originating from vegetables generally have a higher metabolizable energy value as they have higher amounts of unsaturated fatty acids, while animal fats contain higher amounts of saturated fatty acids. Fats and oils from animal and vegetable sources are often blended to produce specific blend of unsaturated to saturated fatty acid ratio resulting in a product with increased metabolizable energy. In this trial two different oil sources were used; refined soya oil and a blend of animal fats and vegetable oils. Both sources are commonly used within the South African broiler industry. Samples of both oils were collected a few weeks before feed formulation and chemically analyzed ensuring accurate AME values was used for both oils during formulation. From the results of the chemical analyses, the AME for young birds (0-21 days) was 36.69 MJ/kg for soya oil while the blended oil was 30.78 MJ/kg; the resulting difference was 5.91 MJ/kg or 16.1% lower for the blended oil. The AME results for older birds (> 21 days) were 37.66 MJ/kg for soya oil and 33.82 MJ/kg for the blended oil, which was a difference of 3.84 MJ/kg or 10.2% lower for the blended oil.

The first objective of the trial was to evaluate if the lysophospholipid used would overcome the reduced AME without any impact on broiler production parameters.These results showed no treatment differences during the first two weeks of the trial. With the addition of LEX, no significant differences between soya oil treatments for live weight were noted, even with the decreased energy values, except for the blended oil with LEX, where a significant lower live weight was observed. Cumulative feed intake was found to be significantly higher for both oils when LEX was added. No significant differences were observed for cumulative mortalities, ADG, PER and EPEF across all treatments. The FCR results showed no significant differences between the three treatments for soya oil, however, the blended oil with additional LEX was significantly lower than both control diets. The results proved the potential to decrease the energy of the feed when adding LEX in the diet without adverse effects on production parameters of broilers.

The second objective was to evaluate if the lysophospholipid used would overcome the reduced AME without any impact on the carcass and organ characteristics. These results showed no effect, positively or negatively on the gizzard, bursa of Fabricius or heart relative weights. However, on the liver as well as the gizzard score the soya oil treatment with added LEX resulted in significantly heavier relative weight and higher score, while the LEX added with the blended oil showed no significant difference between treatments except for a significantly higher relative spleen weight when LEX was added. There is potential to use the

cheaper lower quality blended oil instead of soya oil with no effect on organ characteristics. On carcass characteristics, the addition of LEX showed no difference between treatments, except on the breast portion of both oils with added LEX that were observed to have lower weights than the other treatment diets. Therefore, it can be concluded that adding LEX to the diet of broilers with a decreased AME value, had no adverse effects on the organ or carcass characteristics of the broilers.

Critical findings and possible improvements can include, reducing the AME value by more than the conservative 0.25 MJ/kg done in this trial to obtain an optimum improvement level of the fat emulsifier, also varying both the level of the emulsifier as well as the level of fat included in the diet. Further research and trials are warranted on the use of emulsifiers, as feed is an important cost factor in broiler production. There were not many significant differences observed in the trial, even with reduced dietary energy, the production parameters were higher than the standard for optimal broiler production.